

Supplemental Information

Details of chlorophyll and nitrogen concentrations of central and top two leaves of single cross hybrids of maize during grain filling, pedigree of the hybrids, climate of the growing duration in winter in subtropical foot plain of Himalaya.

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Details of chlorophyll and nitrogen concentrations of central and top two leaves of single cross hybrids of maize during grain filling, pedigree of the hybrids, climate of the growing duration in winter in subtropical foot plain of Himalaya.

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Supplemental Information

Sup Table 1: Details of inbred plant materials and F1 seeds harvested for hybrid trial A1

-----Plantation on Sep 15, 2011-----						--Harvested on March 2012--	
Female parent	Inbred Origin	Pedigree of inbred	Male parent	ORIGIN	Pedigree of inbred	Hybrid	Hybrid entries
RML-19	CIMMYT	CA34501	NML2 OR RML2	CIMMYT	CML 430	RML-19/NML-2	1
RL-137	NEPAL	UPAHAR-B-54-1-1-2-1	RL-168	NEPAL	POOL-21-29-1-1-2-1-1	RL-137/RL-168	2
RML-55	CIMMYT	CML 168	RL-29	NEPAL	na	RML-55/RL-29	3
RL-99	NEPAL	UPAHAR-B-20-2-2-2-1	RL-161	NEPAL	POOL-21-17-1-1-1-1-1	RL-99/RL-161	4
RML-6	CIMMYT	CA34514	RML-19	CIMMYT	CA34501	RML-6/RML-19	5
RL-111	NEPAL	UPAHAR-B-31-1-1-1-1	RL-189	NEPAL	POOL-21-32-1-3-3-2-1	RL-111/RL-189	6
RML-95	CIMMYT	PUTU 17	RML-96	CIMMYT	AG 27	RML-95/RML-96	7
RML-86	CIMMYT	PUTU 20	RML-96	CIMMYT	AG 27	RML-86/RML-96	8
RL-36	NEPAL	na	RL-197	NEPAL	POOL-21-32-1-4-3-3-1	RL-36/RL-197	9
RL-180	NEPAL	POOL-21-32-1-1-2-1-1	RML-5	CIMMYT	CA00314	RL-180/RML-5	10
RML-57	CIMMYT	CLQG-6602	RML-6	CIMMYT	CA34514	RML-57/RML-6	11
RL-170	NEPAL	POOL-21-29-1-1-3-1-1	RL-111	NEPAL	UPAHAR-B-31-1-1-1-1	RL-170/RL-111	12
RL-154	NEPAL	POOL-21-12-1-2-1-4-1	RL-111	NEPAL	UPAHAR-B-31-1-1-1-1	RL-154/RL-111	13
RML-4	CIMMYT	CA00326	NML2 OR RML2	CIMMYT	CML 430	RML-4/NML-2	14
NML 1 or RML1	CIMMYT	CML-433	NML2 OR RML2	CIMMYT	CML 430	Gaurav	15

na = not available

Sup Table 2: Transformation equations from SPAD to leaf chl and N contents

For Estimation of , units	Transformation equations	R ² value	Reference
For leaf nitrogen on % dry weight (1) Preanthesis	$Y = 0.708 + 0.0184 X + 0.0004X^2$	(R ² =0.77)	Dwyer et al., 1995
For leaf nitrogen on % dry weight (2) 2-week AA	$Y = 0.708 + 0.00898X + 0.0004X^2$	(R ² =0.82)	Dwyer et al., 1995
For leaf nitrogen on % dry weight (3) 5-week AA	$Y = 0.494 + 0.026 X + 0.0009 X^2$	(R ² =0.85)	Dwyer et al., 1995
For leaf Chlorophyll content estimation ($\mu\text{g cm}^{-2}$)	$Y = (99 *X)/(144- X)$		Cerovic et al., 2012
^u Where X is SPAD value			
Average temperature for GDD computation	= [(daily maximum+ daily minimum temperature)/2]		McMaster and Wilhelm, 1997

AA = After anthesis

Cerovic ZG, Masdoumier G, Ghazlen NB, Latouche G. (2012) A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf Chlorophyll and epidermal flavonoids. *Physiology Plant*, 146(3): 251–260.

Dwyer LM, Anderson AM, Ma BL, Stewart DW, Tollenaar M, and Gregorich E. (1995) Quantifying the non-linearity in Chlorophyll meter response to corn leaf nitrogen concentration. *Canadian Journal of Plant Science* 75: 179-182.

McMaster, GS & Wilhelm WW. (1997) Growing degree-days: one equation, two interpretations. *Agriculture and Forest Meteorology* 87: 291-300.

Sup Table 3: Characterization of growing duration of the maize hybrids. It was in subtropical winter in Nepal 2012-2013. Base temp is 10⁰C for growing ⁰days computation.

From d-m-year	To d-m-year	DAS days	Degree days	Cum ⁰ Days	Mean Temp	Eff Temp Mean	Av Mx Temp	Av Min Temp	Mx Temp Max	Mx Temp Min	Min Temp Max	Min Temp Min	RH %
4-Oct-12	8-Oct-12	1-5	79.4	79.4	25.9	15.9	32.7	19.0	33.1	32.4	20.8	18.0	93.5
9-Oct-12	18-Oct-12	6-15	144.5	223.9	24.5	14.5	31.3	17.6	31.9	30.7	23.3	14.7	93.5
19-Oct-12	28-Oct-12	16-25	106.3	330.2	20.6	10.6	29.7	11.6	30.8	28.3	14.0	9.0	95.4
29-Oct-12	7-Nov-12	26-35	91.6	421.8	19.2	9.2	29.7	8.6	30.3	29.2	10.2	7.6	94.1
8-Nov-12	17-Nov-12	36-45	75.7	497.5	17.6	7.6	27.6	7.5	28.7	26.7	9.3	6.1	95.9
18-Nov-12	27-Nov-12	46-55	68.2	565.7	16.8	6.8	26.8	6.9	28.8	24.8	8.9	5.6	94.6
28-Nov-12	7-Dec-12	56-65	57.2	622.8	15.7	5.7	25.0	6.5	25.4	24.3	7.7	5.7	95.3
8-Dec-12	17-Dec-12	66-75	51.8	674.6	15.2	5.2	23.2	7.2	24.7	22.2	9.7	5.4	97.1
18-Dec-12	27-Dec-12	76-85	29.5	704.2	13.0	3.0	20.3	5.6	21.8	18.2	8.5	2.2	94.9
28-Dec-12	6-Jan-13	86-95	21.5	725.7	12.2	2.2	22.3	2.1	24.2	18.3	3.3	-0.8	92.2
7-Jan-13	16-Jan-13	96-105	-7.6	718.1	9.2	-0.8	18.8	-0.3	22.4	10.1	1.2	-2.5	93.7
17-Jan-13	26-Jan-13	106-115	25.4	743.5	12.5	2.5	21.2	3.9	24.3	14.9	8.4	1.6	95.4
27-Jan-13	5-Feb-13	116-125	41.7	785.2	14.2	4.2	24.4	3.9	27.2	20.4	9.3	1.7	94.5
6-Feb-13	15-Feb-13	126-135	54.3	839.5	15.4	5.4	24.8	6.0	26.8	20.8	10.1	2.4	92.9
16-Feb-13	25-Feb-13	136-145	70.5	910.0	17.1	7.1	25.5	8.7	28.8	21.5	12.5	4.1	87.6
26-Feb-13	7-Mar-13	146-155	103.0	1013.0	20.3	10.3	30.8	9.8	32.2	29.9	12.6	5.1	89.8
8-Mar-13	17-Mar-13	156-165	123.4	1136.4	22.3	12.3	32.5	12.1	35.2	31.3	13.3	11.1	86.5
18-Mar-13	27-Mar-13	166-175	120.6	1257.0	22.1	12.1	31.6	12.5	33.4	30.4	14.3	10.5	83.4
28-Mar-13	6-Apr-13	176-185	143.0	1400.0	24.3	14.3	34.0	14.6	36.5	31.6	15.7	11.9	82.2

Note: Raw data was taken from National Maize Research Program of Nepal Agriculture Research Council, Rampur, Chitwan, Nepal and processed to formulate the Sup Table 3. Average temperature for GDD computation is equal to [(daily maximum+ minimum temperature)/2] (McMaster and Wilhelm (1997)).

Sup Table 4A: Mean square values of E0 leaf SPAD of the hybrids in the winter trial A₁. It was during the grain filling stage in ten days interval

SOV	DF	Grain yield	E0SP95	E0SP105	E0SP115	E0SP125	E0SP135	E0SP145	E0SP155
REPLICA	2	20.606	33.99	10.59	17.71	20.66	6.16	9.36	140.74
HYBRIDS	14	6.664*	7.622 ns	9.425 ns	12.624ns	11.92ns	15.14ns	24.633**	61.77ns
RESIDUAL	28	3.133	7.57	9.18	9.94	10.52	11.81	9.35	69.62

Sup Table 4B: Mean square values of E3 leaf SPAD

SOV	DF	100%PopSen	E3SP95	E3SP105	E3SP115	E3SP125	E3SP135	E3SP145	E3SP155
REPLI	2	9.49	25.97	6.15	9.64	16.57	24.28	36.33	20.20
HYBRIDS	14	12.022**	28.558**	33.55**	40.24**	44.89**	59.51**	70.34**	126.75**
RESIDUAL	28	3.35	9.90	11.96	13.12	12.67	13.19	16.53	21.27

Sup Table 4C: Mean square values of E0 chl contents in $\mu\text{g cm}^{-2}$

SOV	DF	E095Chl	E0105Chl	E0115Chl	E0125Chl	E0135Chl	E0145Chl	E0155Chl
REPLI	2	105.24	29.93	46.02	50.96	11.28	16.01	227.30
HYBRIDS	14	24.39ns	27.67ns	34.28ns	33.58ns	44.09ns	71.16**	106.4ns
RESIDUAL	28	22.45	25.19	26.69	29.09	31.79	24.78	104.60

Sup Table 4D: Mean square values of E3 chl contents in $\mu\text{g cm}^{-2}$.

SOV	DF	E3-95Chl	E3-105Chl	E3-115Chl	E3-125Chl	E3-135Chl	E3-145Chl	E3-155Chl
REPLI	2	60.24	15.52	22.40	31.66	48.14	70.64	20.60
HYBRIDS	14	60.86**	67.36**	79.25**	86.36**	117.53**	132.74**	193.45**
RESIDUAL	28	23.18	24.63	25.24	24.55	25.97	33.39	33.13

Sup Table 4E: Mean square values of E0 N contents

SOV	DF	1E0 N%PreA	2E095N D	2E0105N D	3E0115ND	3E0125N D	3E0135 N D	E0145N D	E0155 N D
REPLICA	2	0.13	0.09	0.03	0.24	0.26	0.06	0.09	1.13
HYBRIDS	14	0.02955ns	0.02113ns	0.02404ns	0.1712ns	0.1678ns	0.2153ns	0.3484**	0.5413ns
RESIDUAL	28	0.03	0.02	0.02	0.13	0.15	0.16	0.12	0.53

Sup Table 4F: Mean square values of E3 leaf N contents

SOV	DF	1E3 N%PreD	2E3-95N D	2E3-105N D	3E3-115N D	3E3-125N D	3E3-135N D	3E3-145 N D	3E3-155N D
REPLICA	2	0.08	0.05	0.01	0.11	0.16	0.25	0.37	0.11
HYBRIDS	14	0.08308**	0.05689**	0.06335**	0.4231**	0.4561**	0.6162**	0.6924**	1.012**
RESIDUAL	28	0.03	0.02	0.02	0.13	0.13	0.14	0.17	0.17

Sup Table 5A: DMRT of hybrids for leaf chl. Chl content is in $\mu\text{g cm}^{-2}$ among the fifteen maize hybrids

Entries	Popsen100%		^C E095Chl	E0105Chl	E0115Chl	E0125Chl	E0135Chl	E0145Chl		E0155Chl
8	175.33	D	63.00	59.78	59.34	63.30	63.43	61.25	AB	45.27
12	181.67	A	61.61	58.52	57.30	57.41	57.91	59.11	ABC	46.82
11	179.00	ABC	62.46	60.14	56.86	59.31	59.00	66.28	A	55.98
13	180.00	AB	58.15	54.81	50.52	52.30	47.90	48.26	D	34.61
5	178.33	A-D	61.56	56.64	53.72	56.99	54.38	55.29	BCD	42.31
6	178.33	A-D	57.57	58.07	53.55	54.77	51.94	51.20	CD	34.45
10	180.00	AB	63.56	62.31	56.84	57.35	57.06	59.63	ABC	53.32
1	178.33	A-D	64.79	61.09	59.68	56.83	55.91	57.39	A-D	44.54
7	175.67	CD	55.53	53.69	49.15	52.79	54.64	55.62	BCD	41.10
14	176.33	CD	58.80	54.29	55.13	54.32	53.42	55.32	BCD	47.38
2	176.67	BCD	60.60	55.75	52.45	52.46	52.64	51.55	BCD	39.19
9	177.00	BCD	62.34	62.60	55.85	57.10	53.19	56.24	BCD	47.72
15	176.67	BCD	57.14	54.66	52.30	51.98	51.12	52.45	BCD	42.50
4	175.33	D	64.60	58.25	58.35	59.97	57.55	54.82	BCD	46.98
3	175.00	D	59.43	54.42	50.05	52.39	51.43	48.26	D	41.31
Mean	177.58		60.74	57.67	54.74	55.95	54.77	55.51		44.23

^CLeaf chlorophyll concentration was calculated from the transformation equation $Y = (99 * X) / (144 - X)$ which has been given by Cerovic et al. (2012).

Cerovic ZG, Masdoumier G, Ghazlen NB, Latouche G. 2012. A new optical leaf-clip meter for simultaneous non-destructive assessment of leaf Chlorophyll and epidermal flavonoids. *Physiology Plant*, 146(3): 251–260.

Sup Table 5B: DMRT of hybrids for leaf chl. Chl content is in $\mu\text{g cm}^2$ among the fifteen maize hybrids

Entries	E3-95Chl		E3-105Chl		E3-115Chl		E3-125Chl		E3-135Chl		E3-145Chl		E3-155Chl	
8	50.50	A	44.69	ABCD	42.69	BCDE	44.29	CDE	44.42	CDE	43.67	BCD	30.89	DEF
12	50.71	A	53.09	A	51.62	AB	56.27	A	57.56	A	52.50	AB	47.04	AB
11	53.70	A	52.47	A	53.15	A	53.99	AB	56.55	AB	57.03	A	49.62	A
13	49.30	AB	47.65	ABC	46.36	ABCD	48.14	ABCD	47.24	BCDE	48.55	ABCD	40.92	ABCD
5	53.18	A	48.81	ABC	45.59	ABCD	47.45	ABCD	45.57	CDE	46.97	ABCD	42.89	ABC
6	48.56	AB	47.97	ABC	46.02	ABCD	49.07	ABCD	47.30	BCDE	45.53	BCD	37.34	BCDE
10	53.71	A	50.87	AB	47.44	ABCD	47.86	ABCD	47.73	BCDE	47.74	ABCD	38.68	ABCD
1	48.84	AB	43.34	ABCD	42.13	BCDE	40.78	DE	41.21	DEF	39.59	DE	33.73	CDE
7	38.63	C	36.91	D	35.64	E	35.61	E	33.54	F	29.80	E	21.66	F
14	44.39	ABC	42.43	BCD	39.34	CDE	41.17	DE	39.01	EF	37.91	DE	26.53	EF
2	50.82	A	51.32	AB	52.58	A	52.26	ABC	53.29	ABC	51.48	ABC	48.43	A
9	51.88	A	50.57	AB	48.44	ABC	46.28	BCD	49.09	ABCD	44.74	BCD	32.62	CDE
15	40.38	BC	39.71	CD	38.28	DE	41.95	DE	44.09	CDE	40.52	CD	30.91	DEF
4	50.39	A	48.28	ABC	46.45	ABCD	47.08	ABCD	43.99	CDE	40.66	CD	39.44	ABCD
3	48.65	AB	46.80	ABC	47.22	ABCD	47.31	ABCD	45.98	CDE	43.53	BCD	35.06	CDE
Mean	48.91		46.99		45.53		46.63		46.44		44.68		37.05	
LSD	8.05		8.30		8.40		8.29		8.52		9.67		9.63	

^Z Leaf chlorophyll content has been estimated by the transformation equation 'Y = (99 *X)/(144- X)' given by Cerovic et al. (2012).

Sup Table 5C: DMRT of hybrids for leaf N. N is in percent dry weight of ear (E0) leaf sectors of the maize hybrids

Entries	¹ E0NPre-A	² E095N D	² E0105N	³ E0115N	³ E0125N	³ E0135 N	³ E0145N	³ E0155 N	¹ E3 N Pre-A		
8	2.98	2.46	2.36	4.49	4.77	4.76	4.63	AB	3.50	2.54	A
12	2.94	2.42	2.33	4.37	4.38	4.41	4.50	ABC	3.61	2.55	A
11	2.97	2.45	2.38	4.33	4.51	4.49	4.97	A	4.27	2.66	A
13	2.82	2.32	2.22	3.88	4.00	3.69	3.71	D	2.75	2.50	A
5	2.94	2.42	2.27	4.12	4.34	4.15	4.22	BCD	3.30	2.64	A
6	2.80	2.30	2.32	4.10	4.19	3.99	3.93	CD	2.73	2.47	AB
10	3.00	2.48	2.44	4.33	4.37	4.34	4.53	ABC	4.07	2.66	A
1	3.05	2.51	2.40	4.52	4.32	4.26	4.37	ABCD	3.47	2.49	AB
7	2.72	2.24	2.18	3.78	4.04	4.17	4.22	BCD	3.20	2.10	C
14	2.84	2.34	2.20	4.21	4.15	4.09	4.22	BCD	3.65	2.32	ABC
2	2.90	2.39	2.25	4.02	4.02	4.04	3.96	BCD	3.07	2.56	A
9	2.96	2.44	2.45	4.26	4.35	4.08	4.29	BCD	3.67	2.60	A
15	2.78	2.29	2.21	4.00	3.98	3.92	4.01	BCD	3.30	2.17	BC
4	3.04	2.51	2.32	4.44	4.56	4.39	4.19	BCD	3.63	2.54	A
3	2.87	2.36	2.21	3.84	4.01	3.94	3.72	D	3.20	2.48	AB
Mean	2.91	2.39	2.30	4.18	4.27	4.18	4.23		3.43	2.48	
LSD	0.28	0.23	0.25	0.61	0.64	0.67	0.59		1.21	0.30	

⁽¹⁾The same SPADs recorded on 95th day have been transformed into preanthesis nitrogen estimate (Pre-A) by equations of Dwyer et al., 1995
Dwyer LM, AM Anderson, BL Ma, DW Stewart, M Tollenaar, and E Gregorich. 1995. Quantifying the non-linearity in Chlorophyll meter response to corn leaf nitrogen concentration. Canadian Journal of Plant Science 75:- 179-182.

Sup Table 5D: DMRT of hybrids for leaf N. It is mean values of percent dry weight of E3 leaf nitrogen of the maize hybrids

Entries	² E3-95N D		² E3-105N D		³ E3-115N D		³ E3-125N D		³ E3-135N D		³ E3-145 N D		³ E3-155N D	
8	2.09	AB	1.91	ABCD	3.31	BCDE	3.42	CDE	3.44	CD	3.38	BCD	2.47	EFG
12	2.09	AB	2.17	A	3.96	AB	4.30	A	4.38	A	4.02	AB	3.62	ABC
11	2.18	A	2.15	A	4.07	A	4.13	AB	4.31	AB	4.34	A	3.82	A
13	2.05	AB	2.00	ABC	3.58	ABCD	3.71	ABCD	3.64	BCD	3.74	ABCD	3.18	ABCDE
5	2.17	AB	2.04	AB	3.53	ABCD	3.66	ABCD	3.52	CD	3.62	ABCD	3.32	ABCD
6	2.03	ABC	2.01	ABC	3.56	ABCD	3.78	ABCD	3.65	BCD	3.52	BCD	2.92	CDEF
10	2.18	A	2.10	AB	3.65	ABCD	3.68	ABCD	3.67	BCD	3.66	ABCD	3.02	BCDE
1	2.04	ABC	1.87	BCD	3.27	BCDE	3.17	DE	3.20	DE	3.08	DE	2.66	DEF
7	1.72	D	1.67	D	2.79	E	2.79	E	2.64	E	2.38	E	1.78	G
14	1.90	BCD	1.84	BCD	3.07	CDE	3.21	DE	3.05	DE	2.97	DE	2.15	FG
2	2.10	AB	2.11	AB	4.03	A	4.01	ABC	4.08	ABC	3.95	ABC	3.73	AB
9	2.13	AB	2.09	AB	3.73	ABC	3.58	BCD	3.78	ABCD	3.46	BCD	2.58	DEF
15	1.78	CD	1.76	CD	2.99	DE	3.26	DE	3.41	CD	3.15	CD	2.46	EFG
4	2.09	AB	2.02	ABC	3.59	ABCD	3.64	ABCD	3.41	CD	3.16	CD	3.07	ABCDE
3	2.03	ABC	1.97	ABC	3.64	ABCD	3.65	ABCD	3.55	CD	3.37	BCD	2.75	DEF
Mean	2.04		1.98		3.52		3.60		3.58		3.45		2.90	
LSD	0.24		0.25		0.61		0.60		0.62		0.70		0.69	

⁽¹⁾The same SPADs recorded on 95th day have been transformed into preanthesis nitrogen estimate (Pre-A) by equations of Dwyer et al., 1995
Dwyer LM, AM Anderson, BL Ma, DW Stewart, M Tollenaar, and E Gregorich. 1995. Quantifying the non-linearity in Chlorophyll meter response to corn leaf nitrogen concentration. Canadian Journal of Plant Science 75:- 179-182.