Frequency distribution and mean comparisons of red light absorbance-transmittance of the e1 leaf sectors of five pipeline maize hybrids during early grain filling in subtropical winter

Precision of chlorophyll meter measures

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ABSTRACT

Background: Crop scientist's curiosities were to examine variation in photosynthetic sectors of grain filler leaf or flag leaf of any major crop species. Crop scientists including plant breeders too will be privileged from the findings. So it includes such precision evaluation of single cross pipeline hybrids of yellow maize before their release as cultivars. Materials and methods: For it, five pipeline hybrids were examined from two trials of RCBD trial that they were in a big research plot with uniform soil fertility level and with the same control environment. Each of the trials was conducted planting the seeds of the fifteen newly bred separate hybrids on October 3, 2012 exposing their flowering and early grain filling to the natural winter in the subtropical region in Nepal. Five plants of the pipeline hybrids of the fifteen plots of three replications were randomly selected and tagged from each of the fifteen plots of the five hybrids before tassel emergence. After about one month of anthesis, one hundred observations of red light absorbance-transmittance (RAT) measures were taken from chlorophyll meter 'SPAD 502' (Soil Plant Analysis Development) from the just-above-ear (e1) leaf of each of the selected plants of the hybrids in three days from 99 to 101 days after sowing (DAS). Results and discussion: From analysis of frequency distribution of the RAT SPAD measures of the e1 leaf sectors, it is not necessary that hybrids that demonstrates curve with high kurtosis always yield higher. Among the five hybrids, second highest grain yielding hybrid 112 had least leaf sectors of very low RAT SPAD measures. Besides, the hybrid 112 has been found least skewed and least peaked among the five hybrids. Furthermore, the hybrid 112 displayed least coefficient of variation and standard deviation among the 1500 RAT SPAD readings of its e1 leaf sectors. From the mean analysis through DMRT (Duncan Multiple Range Test), the twenty four averages have been found almost non-significant different among themselves inside each of the five hybrids. Single

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reading and four reading averages were non-significant different to one hundred reading average of each of the fifteen selected plants. Besides, the study also implies that a single reading from a leaf in the surrounding region of the topmost ear in five randomly selected plants can suffice to reflect the average SPAD measure of the particular leaf if the readings are from the e1 leaf. But, it can also be concluded that the similar non-significant nature of average central leaves around the top ear node. The mean comparison also gives useful information about the methodology of taking SPAD observations and how many readings are enough for the study of red light absorbance-transmittance, chl and N concentration in maize leaves. It also includes explanation why the e1 leaf is taken in the study.

Subjects: plant genetics, crop physiology, plant breeding, soil-plant relation, plant optics

Keywords: maize leaf chlorophyll, maize leaf N concentration, maize leaf SPAD measure, red light absorbance-transmittance, RAT, *Zea mays* L., DMRT, Duncan Multiple Range Test, Soil Plant Analysis and Development, anthesis, tassel emergence,

INTRODUCTION

Some single cross hybrids of yellow maize have reached on cultivar release stage in national maize breeding station in Nepal with efforts of maize breeding scientists. Generally, newly bred pipeline lines are examined from different perspectives in the crop breeding institute before their final release. Leaf manufactures food for plants through photosynthetic apparatuses including chlorophyll (chl) pigment. In order to examine the photosynthetic sectors of the one of the largest assimilate contributing leaves: e1 (just above ear leaf) in general maize hybrid (Subedi and Ma, 2005), red light absorbance-transmittance (RAT) measure of green pigments (chl) has been used. It is because red light is most efficiently utilized in leaves than blue light; but blue light is absorbed by flavonoids in vacuoles and carotenoids in thylakoid (Inada 1976). Green light in white light from the sun is as efficiently utilized as the red light by the green pigments of shallow as well as deep leaf mesophyll cells (McCree 1972; Inada 1976; Gates 1980; Bjorkmann 1968; Balegh and Biddulph 1970; Terashima et al., 2009).

Since RAT measure can be index for measure of photosynthetic efficiency of leaves, the measure can imply about the photosynthetic efficiency of the leaves including grain filler leaf around the ear or just above the ear. The RAT measure taken from a device of chlorophyll meter SPAD 502 (SPAD-502 Konica Minolta Sensing Inc., Japan) gives unit less SPAD (Soil Plant Analysis Development) measure (Minolta, 1989). We can say it RAT SPAD measure or SPAD which is based on the absorbance and transmittance of two wave lengths of light: red (of 650 nm) and infrared light (of 940 nm) by the leaf tissue especially chl (Markwell 1995). Several plant scientists have used the device to measure leaf chl conc of rice, maize, wheat and some more crops.

The SPAD device has been used to estimate leaf N conc. Murdock et al. (1997) reported that the SPAD measure is proportional to leaf N and chl conc. Yadava (1986) reported that the leaf SPAD observations have been found linearly correlated with leaf chl conc for several crops. Cerovic et al., (2012) discovered more refined formulae to estimate total leaf chl conc in leaves of higher plant species from SPAD measure than the chl extracted in most laboratories in the world. Escobar-Gutierrez and Combe, (2012) too discovered of similar relationship between RAT SPAD measure and chl conc. Toth et al., (2002) showed growth of carotenoid and chl conc in maize leaves takes place in response to N fertilizers and they effect on increased CO_2 fixation and grain yield. Netto et al., (2002; 2005) demonstrated sigmoid (curvilinear-plateau) correlation of leaf RAT SPAD measure to photosystem II (PS II) based photosynthetic electron transport (PET) Fv/Fm with R-square greater than 80%.

Several plant and crop science related scientific papers have included the SPAD measure to characterize crop genotypes for several purposes. No any paper has demonstrated about the variation among the mean values of the photosynthetic sectors of the same leaf from the device. In other words, no one reported how uniform or differences are in different region of a single lasting e1 leaf of maize from the standpoint of chl and N concentration. This paper has dug into this aspect. It includes the significance of photosynthetic sectors of grain filler e1 leaf. It also include number of readings of the chlorophyll meter or RAT measure required for a single mean to study the photosynthetic potentiality of the leaf for the crop of maize.

MATERIALS AND METHODS

Characterization of the growing period

Location of the research site is in the longitude $27^0$37'N, latitude $84^0$24'E and altitude 228 m above sea level. Soil is sandy loam and pH in the range of 5-5.5. Daily temperature data were taken from meteorology division of the NMRP / NARC Nepal. Climate of the research site is characterized as subtropical; the winter is characterized as suboptimal chilling night during flowering to mid grain filling. The climate data of crop growth period from sowing to till the crop was harvested have been summarized and presented by Adhikari et al. (2015).

Materials and planting

The experiment was conducted at the breeding station of National Maize Research Program of Nepal Agriculture Research Council, Rampur, Chitwan, Nepal which is located on $27^0$37'N as longitude and $84^0$24' E as latitude and topography is 228 meter above sea level. The soils have traits of sandy loam texture and 5.0 to 6.5 pH. The two hybrid trials were conducted in a big research plot of National Maize Research Program (NMRP) in Nepal Agriculture Research Council (NARC) in winter season of 2012-13. The two trial plots were adjacent. The soils of the trial plot were uniform from the standpoint of soil fertility, soil texture, soil moisture level and openness. The control condition, fertilization, irrigation and intercultural operations were the same for both the trials. The pipeline hybrids selected are RL-111/RL-189 (6), RML-32/RML-17 (112), RML-4/NML-2 (14), RML-4/RML-17 (113) and RC/RML-8 (109) with their entry number and they were in variety release process.

Planting: Compost was applied at rate of 33 t ha^{-1}. The field was ploughed and made clod free. Fertilizer was applied @ 120:60:40 kg N, P_2O_5 and K_2O ha^{-1}. In basal dose, 50% N, all phosphorus and potassium fertilizers were applied through DAP and murate of potash. Remaining 50 % was applied in split dose as top dressing at rate of 25% in the form of urea on 45 and 60 days after sowing (DAS). Planting was manually done on October 03, 2012 to expose flowering and grain filling to natural chilling winter to mimic the commercial maize growing season in granary belt of Nepal. Rows in each block were continuous; but they were separated by 0.70 m. Blocks were separated with an alley of 1 m. The row direction was on north-south. Two seeds were dropped on 0.25 m spacing using a simple seed sowing lever along each 3-meter long row of each plot of 3 x 1.4 m^2. Soil loosening and weed removal was done manually on 30 DAS and twenty four plants were maintained in each plot on 30 DAS to keep plant density @ 57,143

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plants ha^{-1}. Earthing-up was done on 45 DAS. Plants were irrigated through furrow on 50, 70, 90 and 110 DAS through shallow tube well of 4" pipe. The crop was harvested on 185th day.

Morpho-physiological traits

Days to tassel emergence, anthesis and silk emergence in the earliest 50% population have been recorded from each of the fifteen plots. Ear height and top height were measured from each of the randomly selected five plants from each plot. Then culm length was measured as the sum of the ear height and top height up to the terminal node of tassel emergence. Grain yield in t ha^{-1} was determined adjusting moisture content at 15% for each plot separately. Anthesis-silking interval (ASI) was computed subtracting anthesis days from silking days and tassel emergence-anthesis interval (TAI) was computed subtracting days of tassel emergence from the days for anthesis. Here, the data of the anthesis, silk initiation (silking) and tassel emergence implies for days of expression of the traits in the earliest 50% population in each plot. The data were analyzed through F-test and DMRT techniques to determine the validity of difference among the pipeline hybrids (Table 1). The traits of ASI and TAI have not been examined through F-test and DMRT.

Measure of photosynthetic sectors through red light absorbance on the e1 leaf

RAT SPAD measures were taken through SPAD 502 from both sides of the mid rib of the e1 leaf from each of the fifteen plants in three plots of each of the hybrids. The RAT SPAD measurements were taken in the region just above the 1.5 cm leaf margin of the leaf with a SPAD-502 chlorophyll meter in three-day period from 99 to 101 days after sowing (DAS). One hundred observations of the SPAD were taken from each e1 leaf. Altogether fifteen observations have been taken from the fifteen plants of each of the five hybrids on 99 to 101st day after sowing. Seventy five row data of the randomly selected seventy five plants of the fifteen plots of the five pipelines were made ready in Microsoft Excel Worksheet.

Computation of averages, variance analysis and mean comparison

Three types of averages of the photosynthetic sectors of the e1 leaf have been computed. They are four-reading, single reading and 100-reading averages of the RAT SPAD observations from each of fifteen plants. In order to compute four reading average; first four continuous readings in each plant row were used to compute the average. After five cells in the row, then next continuous four reading average was computed from the same plant row starting from sixth column of the row. After ten cells in the row, then next continuous four reading average was computed from the same plant row starting from eleventh column to fourteenth of the row. This way plant average is computed. Then plot averages were computed from the five plants of each plot. This way twelve four- reading average were computed from 48 columns out of continuous 60 columns. Here, one cell is left out of each five cells in each row plant in four reading average.

For eleven averages of single reading; single reading of five plants in each plot was used to compute plot average after 60 columns of each plant row. And third and last average is of 100 readings of each of the five plants were computed in its row, then mean from the five-plants was used to compute 100 reading plot averages. The twenty four averages of the SPAD measure were analyzed using two-way RCBD variance analysis using plot average value defining hybrids as factor A (5) and number of averages as the factor B (24). DMRT table was constructed to compare the averages of the photosynthetic sectors of the e1 leaf through the RAT SPAD measures (Table 4).

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RESULTS

Characterization of the hybrids from general traits

Among the five single cross hybrids of the yellow maize, the lowest grain yielding hybrid 109 is a top cross hybrid made crossing between an open pollinated maize cultivar: Rampur Composite (RC) and an inbred line. Other four hybrids are single cross hybrids made crossing two inbreds based on previous evaluation at NMRP/ NARC. Three highest grain yielding hybrids were 6, 112 and 14. Medium grain yielding hybrid was 113 and lowest yielder was 109. But the hybrids were non-significant different from the standpoint of grain yields (Table 1). Culm length, days for initiation of anthesis, initiation of silk emergence of the earliest 50 % population and days for senescence of the 100% population have been significant different among the hybrids through F-test and DMRT. Highest and lowest grain yielding hybrids 6 and 109 respectively were tallest and they have been found significant different from the remaining three hybrids 112, 14 and 113 from the standpoint of plant height. The hybrids 6 and 109 displayed wider TAI and ASI than that of the remaining three hybrids (Table 1).

Table 1: General morphological, physiological and yield traits of the pipeline hybrids

†Hybrid Entry	[1]Yield (t ha^{-1})	[2]Culm Len (cm)	[3]Anth50 (days)	[4]Silk50 (days)	[5]Pop Sen (days)	[6]TAI (days)	[7]ASI (days)	[8]SPAD35 (%)
6	11.02 A	190.3 A	74.3 C	78.3 BC	178.3 B	4.7	4.0	6.9
112	10.26 A	158.7 B	78.3 B	79.3 B	181.7 A	0.7	1.0	4.0
14	9.64 A	164.8 B	82.0 A	83.0 A	176.3 B	2.3	1.0	7.5
113	8.90 A	161.2 B	84.3 A	84.3 A	181.3 A	2.7	0.0	6.5
109	7.52 A	197.2 A	71.3 D	76.7 C	175.7 B	4.3	5.3	9.1

†Hybrids and their entries are RL-111/RL-189 (6), RML-32/RML-17 (112), RML-4/NML-2 (14), RML-4/RML-17 (113) and RC/RML-8 (109). [1]Grain yield, [2]Culm length; [3]days for anthesis of 50% population; [4]days for silk emergence of the 50% population; [5]days for senescence of the all plants in the plot of each hybrid; [6]tassel emergence-anthesis interval in days; [7]anthesis-silking interval in days; [8]percent of the frequencies of the e1 leaf sectors of SPAD below 35. Grain yield has been determined at plant population density 57,143 plants ha^{-1}

Frequency distribution of e1 leaf sectors

FD of the e1 leaf sectors of the hybrid 6 has been found taller (leptokurtic almost equal to 4) among the five pipelines. It cannot be concluded that the highest grain yield in the hybrid 6 is because of the highest maximum RAT SPAD measure among the five hybrids (Table 3). Second highest grain yielding hybrid 112 (RML-32/RML-17) is characterized as having FD of e1 leaf RAT SPAD measures as slightly skewed towards left and least peaked among the five hybrids. Here, we cannot conclude that the traits of FD of the e1 LS favored the hybrid 112 for its high grain yield relatively in winter in the subtropical region in Nepal. Third highest grain yielding hybrid 14 (RML-4/ NML-2) demonstrated highest negative skewness and peakness of 3.69 in the FD of e1 leaf sectors from the standpoint of the RAT SPAD measure. The fourth medium grain yielding 113 (RML-4/RML-17) displayed FD with kurtosis of almost equal to 4 and second high skewness towards left based from the standpoint of the RAT SPAD among the five hybrids. And highest kurtosis observed on the hybrid 113 among the five pipelines was not proportionately involved to produce parallel grain yielding with the three highest grain yielding hybrids.

In addition; from the standpoint of varying chl conc, FD of the hybrid 113 has been found left skewed (-0.52) and less peaked (kurtosis of 1.38). Here; it can be discussed that the hybrid 113 could not utilize leaf red light absorbance as efficiently for grain yield increment as the hybrid 6. The hybrid may have high potentiality of photochemistry; but it could not produce high grain yield proportionately. FD of the lowest grain yielding hybrid 109 (RC/RML-8) is characterized as having kurtosis of 2.62 from standpoint of the SPAD. FD is less peaked than normal (Table 3). In hybrid 109, average SPAD just above of the threshold has not been found proportionately involved in the production of comparable grain yield with other four hybrids (Table 1, 2 and 3). But; the low SPAD average is not really low, it is non-significant different from the other four hybrids.

Comparison among single, four reading and one hundred reading RAT SPAD averages

In many original research papers published in several peer reviewed international journals of applied plant science, SPAD 502 reading have been extensively used to imply N and chl conc of leaves. So how each reading of SPAD varies to next reading must be examined for the reliability of the procedure of the observation taken through the SPAD 502 device.

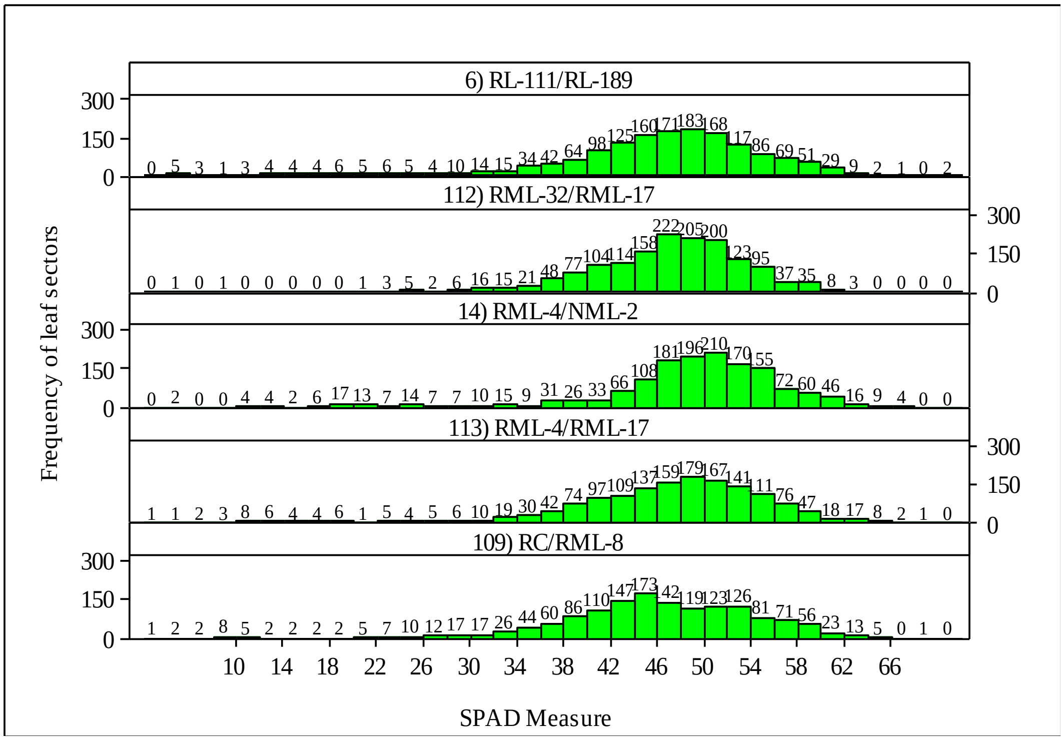

Figure 1: Frequency distribution of e1 leaf sectors based on varying RAT SPAD measures. The Frequency distributions were plotted taking 1500 observations on the fifteen e1 leaves from each of five maize hybrids including recently released Rampur hybrid 2 (RML 4/NML2). The SPADs were recorded in three days continuously from 99 to 101 days after sowing.

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Table 2: Frequency distribution of e1 leaf sectors of varying SPAD measure of the five hybrids

[1]Hybrid entry	Mean	Trimmed Mean	Standard deviation	Coefficient of variation	Min	Max	Skewness	Kurtosis
6	46.6	47.2	8.68	18.61	5	71.3	-1.38	4.09
112	47.0	47.2	6.46	13.75	5.9	62.7	-0.82	2.34
14	48.4	49.3	9.07	18.71	4.5	67.6	-1.62	3.69
113	47.0	47.7	8.82	18.76	2.2	68.8	-1.43	4.07
109	45.7	46.2	9.15	20.04	3.2	69.0	-1.06	2.62

[1]The mean values were computed from 1500 observations for each hybrid. Hybrids and their entries are RL-111/RL-189 (6), RML-32/RML-17 (112), RML-4/NML-2 (14), RML-4/RML-17 (113) and RC/RML-8 (109).

In most of the works published earlier by other plant scientists using SPAD 502; four to five readings have been taken for mean comparison in a variety of study. So, what about the reliability of the four readings is have been compared with single reading as well as one hundred reading averages inside a single central leaf through variance analysis and mean comparison using the DMRT. In table 5; in the column of the highest grain yielding hybrid 6, twenty two averages have been found non-significant different among themselves except two out of 24. In the second highest grain yielding hybrid 112; twenty three averages out of 24 has been found non-significant different. In medium grain yielding hybrid 14; all averages have been found non-significant different among the 24. In fourth grain yielding hybrid 113; twenty out of the 24 averages have been found non-significant different. In the lowest grain yielding hybrid 109; nineteen averages have been found non-significant different among the twenty four.

Non-significance or significance of mean values of RAT SPAD of the same leaf varies from genotype to genotype. In hybrid 14; non-significance of all the averages of the RAT SPAD indicates that even a single reading average is enough if the averages of the same leaf have been compared. And; majority of the averages of the four hybrids except 109 has been found non-significant different. This sort of analysis indicates that; if the RAT SPAD observations of a kind of a leaf of a genotype have to be taken; mean value computed from one reading from each of five plants is enough for the RAT SPAD average. But; extreme aberrant SPAD reading must be eliminated. In addition; RAT SPAD reading should be taken from the representative green region of the leaf. Furthermore; it can be stated that a single reading is enough to explain RAT SPAD if RAT SPAD reading is properly taken from one kind of a leaf (Table 3 & 4).

Majority of the e1 leaf sectors had RAT SPAD measure above 35 in the peak grain filling. So, SPAD 502 must be handled for taking the observation omitting outliers if few observations have to be taken. Otherwise; many RAT SPAD observations have to be taken for correct RAT SPAD average. In essence; the few reading average will not be free from errors if outliers are not eliminated. So outliers must be eliminated. Here; in the study, every continuous 100 readings have been included into data analysis spreadsheet for precision evaluation of the pipeline hybrids.

Trimmed and untrimmed means from 100 SPAD observations have been shown in table 2. When the averages are compared with the mean values in DMRT table 4, the averages inside the same hybrid have been found non-significant different with the majority of four-reading, single reading and 100-reading averages (Tables 2 & 4). Trimmed mean of SPAD of each of the five hybrids have been found very close to untrimmed mean. Non-significant differences among the 24 different kinds of e1 leaf SPAD averages extracted from the variance analysis also says that

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averages obtained from a single reading, four reading and one hundred reading of the SPAD obtained from the same e1 leaf of the fifteen randomly selected plants of 3 replications of the same hybrid convey the similar aroma since all sort of averages inside the genotype of the maize were non-significant different (Table 4).

In the hybrids; hardly 1% SPAD observations have been found outliers. The e1 leaf SPADs below 10 have been considered as outliers in the distribution (Fig 1). It means that there is 1% possibility of mistakes of taking SPAD on central leaves especially e1 in maize when single cross hybrids were at peak grain filling stage and only few readings have been taken for study.

Table 3: Variance analysis of the SPAD measure of e1 leaf of pipeline hybrids as factor A and type of averages as factor B.

SOV	DF	Sum of Square	Mean Square	F VALUE	PROBABILITY
REPLICATIONS	2	761.5	380.75	28.76	
HYBRIDS (A)	4	221.56	55.39**	4.18	0.003
AVERAGES (B)	23	957.83	41.64**	3.15	<.001
A x B	92	1138.39	12.37 NS	0.93	0.641
RESIDUAL	238	3150.43	13.24		

Then Variance analysis table has been constructed defining hybrids as factor A (5) and averages as factor B (24).

Table 4: SPAD mean comparison by Duncan Multiple Range Test (DMRT). SPAD means are from four readings (Rd4), single reading (Rd1) and 100 reading (H) taken on the e1 leaf of each of the five plants from each of the fifteen plots of the five pipelines.

Hybrids	|-------6--------|		|------112------|		|-------14-----|		|-------113-------|		|-------109-------|	
$SPAD_{\mu}$	[1]Rd4	**Rd1,<u>H</u>**	Rd4	**Rd1,<u>H</u>**	Rd-4	**Rd1,<u>H</u>**	Rd4	**Rd1,<u>H</u>**	Rd-4	**Rd-1,<u>H</u>**
1, **13**	48 ab	**50 a**	42 b	**49 ab**	46 a	**48 a**	42 e	**47 a-e**	50 ab	**50 ab**
2, **14**	49 ab	**48 ab**	47 ab	**49 ab**	48 a	**48 a**	46 a-e	**51 a**	51 a	**50 ab**
3, **15**	49 ab	**49 ab**	48 ab	**47 ab**	50 a	**49 a**	49 a-d	**50 abc**	50 ab	**49 abc**
4, **16**	48 abc	**49 ab**	51 a	**49 ab**	49 a	**47 a**	50 abc	**51 ab**	49 abc	**47 abc**
5, **17**	48 ab	**41 c**	48 ab	**47 ab**	48 a	**47 a**	51 ab	**50 abc**	48 abc	**44 a-d**
6, **18**	46 abc	**44 abc**	49 ab	**47 ab**	49 a	**50 a**	51 a	**44 b-e**	46 abc	**44 a-d**
7, **19**	44 abc	**48 ab**	47 ab	**46 ab**	49 a	**46 a**	50 abc	**42 de**	44 a-d	**46 abc**
8, **20**	42 bc	**46 abc**	45 ab	**50 a**	48 a	**51 a**	48 a-e	**47 a-e**	42 cd	**47 abc**
9, **21**	43 abc	**47 abc**	44 ab	**46 ab**	47 a	**50 a**	45 a-e	**47 a-e**	39 d	**44 a-d**
10, **22**	44 abc	**46 abc**	45 ab	**47 ab**	48 a	**52 a**	45 a-e	**45 a-e**	43 bcd	**47 abc**
11, **23**	44 abc	**46 abc**	45 ab	**49 ab**	48 a	**47 a**	43 cde	**49 abc**	43 bcd	**42 cd**
12, **<u>24</u>**[HU]	46 abc	**<u>47 abc</u>**	46 ab	**<u>47 ab</u>**	47 a	**<u>48 a</u>**	44 a-e	**<u>47 a-e</u>**	45 a-d	**<u>46 a-d</u>**

DMRT has been determined for each pipeline hybrid using error mean square and error degree of freedom through MSTAT as shown.

Means in normal font are from four readings (1 to 12 from four Rd4) for each hybrid column and **bold font** ones are from one reading (13 to 23 for Rd1) and **<u>underlined bold ones</u>** are for 100 readings (24 for 100Rd) averages in two columns.

[1]Rd for Reading(s), [HU]One hundred readings. [**U**]Underlined averages are of 100 readings of SPAD observations.

DISCUSSION

SPAD measure expression on suboptimal temperature

Duration from the first to fifth day inside the 95-105 DAS (before SPAD recording) has been characterized with min / max temperature -0.30/18.8^{0}C (Adhikari et al., 2015) since the RAT SPAD recordings were done on 99 to 101 days after sowing. Emergence and development of the e1 leaf happened after 40 days after sowing. The average minimum temperature remained in the range from -0.30^{0}C to 8.6^{0}C in the days from 26 to 105 days after sowing (Adhikari et al., 2015). This means that minimum temperature worked as suboptimal chilling that influenced the development of the leaves above the topmost ear leaf. So; the low RAT SPAD measure in the e1 leaf is the combined effect of chilling and level of tolerance of the genotype of the maize hybrids under adverse biotic and abiotic stresses. The second highest grain yielding hybrid 112 displayed frequency distribution of the leaf SPAD distribution in such a way that the standard deviation and coefficient of variation of the 1500 SPAD observations of the fifteen selected plants have been found lowest among the five hybrids. It means that the photosynthetic apparatus of the e1 leaf of the hybrid 112 were least damaged by the chilling temperature in the winter. Jompuk et al. (2005) too showed a table in their paper that reflects chilling tolerant maize genotypes had higher SPAD than that of sensitive ones. Similarly; according to them, PET rate reflecting Fv/Fm measure and quantum yield implying ΦPS II were also found higher in the chilling tolerant than in sensitive ones in the young maize plants (Jompuk et al., 2005).

Why e1 leaf for the study?

Some discussions about reasons of selecting the e1 leaf in the study have been done here. Bottom leaves indicate about the strength, health and performance of roots whereas leaves above and around the ear node indicates strength of the leaf to contribute to plant dry matter including grain yield (He et al., 2003; Yan et al., 2011). The e1 leaf is the central leaf that stays green longer than most of the top and bottom leaves which can be reflected from bidirectional plant senescence in the maize (Tollenaar and Daynard, 1978; Wolfe et al., 1988). Third important reason is that the ear (e0) leaf is badly damaged by the large size pointed ears of most of the promising hybrid of the maize. Observations of one hundred RAT SPAD measures from the damaged e0 (topmost ear) leaf is not of much practical and logical. So the e1 leaf has been selected for the RAT SPAD observations. In the work, only e1 leaf is examined. The e1 leaf reflects central leaf of the maize around ear, leaves around ear is significant grain filler (He et al., 2003; Yan et al., 2011). Furthermore, In maize, photosynthetic potential and efficiency of mobilization of photo assimilates of the different leaves depends on genotypes (Subedi and Ma, 2005), plant density (Pendleton and Hammond, 1969; Subedi and Ma, 2005), genotype x environment interaction (Tollenaar, 1977) and grain yield is determined by number of leaves below and above the ear in the plants (Subedi and Ma, 2005), kernel numbers per plant (Andrade et al., 1999), position of the ear (Shaver, 1983; Dijak et al., 1999; Subedi and Ma 2005; Pendleton and Hammond, 1969). Tollenaar (1977) suggested that leaves below ear supply assimilate to roots and lower internodes; but leaves above the ear mobilize nutrients to ear for grain fill.

There is no report to reflect about pattern of the N and chl of the grain filler leaf on any date during peak grain filling based on the RAT SPAD measures in maize. The e1 leaf can be representative of all the central five leaves (ear leaf, two leaves below topmost ear node and two leaves above the ear node) which needs further study for the verification.

Abbreviations
SPAD: Soil Plant Analysis Development; chl: chlorophyll; N: nitrogen, e1 or E1 leaf: first leaf just above the ear; RCBD: Randomized complete block design; TAI: tassel emergence-anthesis interval; SAI or ASI: anthesis-silking interval; RAT: Red light absorbance-transmittance (implying); RATA: Red light absorbance and transmittance in antenna region pigments; LS: Leaf sectors;DAS: days after sowing; PET: photosynthetic electron transport; Fv/Fm: variable fluorescence / maximum fluorescence; DMRT: Duncan's multiple range test; r: Pearson's pairwise linear correlation coefficient.

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ADDITIONAL INFORMATION AND DECLARATIONS

Competing interests
Authors declare there have no competing interests.

Patent: Information included in text, tabular and graphical forms are the patent of Dr. N R Adhikari (2015) in PeerJ Preprint sent on October 30, 2015 for average and frequency distribution of SPAD or red light absorbance-transmittance observations and their relationship to grain yield of the maize in any forms in preprint topic "**Frequency distribution and mean comparisons of red light absorbance-transmittance of the e1 leaf sectors of five pipeline maize hybrids during early grain filling in subtropical winter**."

Author's contributions
NRA conceived the concept, conducted experiment, collected data, entered and analyzed data, tabulated the analyzed data, wrote the paper. **SKG** corrected and approved the concept. **SKS** approved the concept. **KBK** worked in the development and maintenance of inbreds, development of materials, conducted experiment, commented on the manuscript. All authors approved the final manuscript.

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