Discovery of a few photosynthesis models through red light absorbance-transmittance of the e1 leaf sectors of newly bred hybrids of maize (*Zea mays* L.)

Nav Raj Adhikari[1*], Surya K. Ghimire [2], Shrawan Kumar Sah[3] and Keshab Babu Koirala[4]

[1]Department of Plant Breeding, Institute of Agriculture and Animal Science, Tribhuvan University, Chitwan, Nepal.

[2]Department of Plant Breeding and Genetics, Faculty of Agriculture Agriculture and Forestry University, Rampur, Chitwan, Nepal.

[3]Department of Agronomy, Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal

[4]Planning Division, Nepal Agriculture Research Council (NARC), Singhdarawar Plaza, Kathmandu, Nepal.

*Corresponding author: navraj.adhikari@gmail.com

ABSTRACT

Background: Pipeline hybrids of maize must be evaluated very intensively and from several perspectives in order to extract reliability of success of the hybrids in farmers' field before their release as cultivars and to extract some useful physiology preferably photosynthesis, source-sink relationship and tolerance of green LS (leaf sectors) under biotic and abiotic stresses as scientists' bonus. For it, five pipeline single cross hybrids of yellow maize have been examined from the standpoint of photosynthetic potentiality of central leaf and nutrient mobilization from it to ear for grain fill. **Materials and methods**: Just above ear (e1) leaf have been minutely examined through ratio of absorbance to transmittance of red light of 650 nm (RAT) measure, chl and N concentration (conc). From correlation coefficients (r) computed between frequency of LS of varying RAT SPAD (Soil Plant Analysis and Development) measures, chl and N conc; few photosynthetic models have been proposed to explain leaf physiologies and its effects on grain yields. **Results and discussions**: Through analysis of frequency distribution of RAT measures and r between frequency of LS of varying RAT SPAD measure and grain yield, RATA Makai model has been proposed to explain for a variety of efficiency of antennae to harvest light energy especially red photons and transfer the energy to reaction centers (RCs). It includes two types of classification of antenna. Next is diversity model to explain existence of four types leaf sector such as narrow, medium, wide diversity with least bad LS in central leaf of the maize based on photosynthetic efficiency of variety of the photosynthetic e1 LS that they are

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differentially contributing to grain yields. This sort of LS diversity study helps classify character of LS based on RAT and or chl and N conc. A few more models proposed in it are NARC (Nitrogen Concentration in Antenna and Reaction Centers) and PAY (Protein Amounts and its effects on Yield) based on r between grain yield and leaf N and protein amounts. In addition; strong positive r has been found between grain yields and the frequencies of the LS of 46-52 RAT SPAD; 46-56 µg cm^{-2} of chl conc and 2-2.3% N of dry weight; This way, we proposed about threshold and upper limit of the RAT SPAD, chl and N conc in maize leaves. It implies that cells in these ranges are genotype based multiplicative and physiologically active and competent to make their identity in the e1 leaf of the hybrids for GY contribution. In addition, next model of 'green car' with the four kinds of proportion of green and carotenoid (car) conc: high green-high car to produce high grain yield and remaining proportion such as high green-low car, low green-high car and low green-low car on maize leaves to cause low grain yield has been proposed. Concept of high green-low car conc in maize leaf gives extended model of no car-no food.

Keywords: red light absorbance, red light absorbance-transmittance, Soil Plant Analysis and Development, RAT SPAD, threshold chl, threshold N conc, threshold SPAD, upper boundary of RAT SPAD, chl and N concentration. RATA Makai Model, Diversity model, N in antenna and reaction center (NARC) model, Protein amounts and its effects on yield (PAY) model, Green car model, and no car-no food.

List of abbreviations used
ARC: Antenna and reaction center; ASI: anthesis-silking interval; chl: chlorophyll; DAS: days after sowing; e1 or E1 leaf: first leaf just above the ear; EE: excitation energy; FD: frequency distribution; FEET: (Förster's) Fluorescence Excitation Energy Transfer; FLSSR: Frequencies of leaf sectors in the SPAD range; FLSNR: Frequencies of the leaf sectors in the N range; FLSCHLR: Frequencies of the leaf sectors in the chlorophyll range; LSNR: Leaf sectors in the nitrogen range; car: carotenoid; cars: carotenoids; Fv/Fm: variable fluorescence / maximum fluorescence; GY: grain yield; LS: Leaf sectors; LSSR: Leaf sectors in the SPAD range; LSCHLR: Leaf sectors in the chlorophyll range; N: nitrogen; NARC: Nitrogen concentration in antenna and reaction centers; NPQ: non-photochemical quenching; PEPC: phosphoenol pyruvate carboxylase; PAY: Protein amounts and its effects on yield; PET: photosynthetic electron transport; PQ: Plastoquinone; qP: photochemical quenching;PA: peripheral antenna; PODK: pyruvate orthophosphate dikinase; PS: Photosystem; PS I: Photosystem I; PS II: Photosystem II; r: Karl Pearson's pairwise linear correlation coefficient; RAT: Red light absorbance-transmittance (implying); RATA: Red light absorbance and transmittance in antenna region pigments; RC: Reaction center; RC II: reaction center II of PS II; RCBD: Randomized complete block design; RUBISCO: Ribulose 1,5-bisphosphate carboxylase oxygenase, SPAD: Soil Plant Analysis Development; Sup file: Supplementary file; Sup table: Supplementary table in Sup file; TAI: tassel emergence-anthesis interval.

INTRODUCTION

Red light absorbance-transmittance (RAT) has been measured through chlorophyll meter 'SPAD-502' (SPAD-502 Konica Minolta Sensing Inc., Japan) (Minolta Camera Co Ltd, 1989). The device is self-calibrating and quickly measuring portable device that gives unitless reading for leaf greenness without leaf destruction. The device emits two wave lengths of light: red (of

650 nm) and infrared light (of 940 nm) and gives reading for ratio of absorbance to transmittance of the two waves The reading given by the SPAD implies total amount of chl a and chl b in thylakoid membrane in the leaf mesophyll chloroplasts. Simultaneously, the device has been used to estimate leaf N conc from the SPAD measure.

Stocking and Ongun (1962) and Hageman (1986) reported that 50 to 70% of maize leaf N is associated to chloroplast. Murdock et al. (1997) reported that the SPAD measure is proportional to leaf N and chl content. Yadava (1986) reported that the leaf SPAD observations have been found linearly correlated with leaf chl conc for several crops. The SPAD measure does not give reading of the leaf N content directly since larger part of N is in nitrate form and is not associated to leaf chl. There is nitrate as well as amino forms in protein and enzymes complexes in green leaves in addition to majority forms of N in the leaf chloroplast embedded chl (Stocking and Ogun, 1962; Hageman, 1986). Dwyer et al. (1991; 1995) displayed that the SPAD measure at central leaves is correlated to the leaf N content in slightly quadratic pattern in maize. Wood et al. (1992) reported that the typical curvilinear relationship was observed between grain yield and leaf N content. Cerovic et al. (2012) and Escobar-Gutierrez and Combe, (2012) reported refined formulae to transfer RAT SPAD measure to total leaf chlorophyll conc. Toth et al. (2002) showed that growth of chl and carotenoid (car) conc in maize leaves in response to nitrogen fertilizers application.

N is in photosynthetic apparatuses such as antenna, reaction center (RCs) chl in thylakoid bilipid membranes in chloroplasts; enzymes, proteins in M (mesophyll cells) and BSCs (bundle sheath cells). Under optimal environment, the leaf photosynthetic apparatus such as pigment-protein complexes, PS II (photosystem II), plastoquinone pool (PQ), electron donors and recipients, PET chain, ATP synthase, NADP reductase and PS I participate efficiently in the phenomena of harvest light energy and transfer the excitation energy (EE) to the RC. Around PS core, PA pigment protein complexes harvest light energy, pigment electron becomes excited, EE migrates from pigment to chl through Forster's FEET (fluorescence excitation energy transfer 'resonance') and then finally reach PS II RC chl a (Forster 1948). Ejection of electrons takes place from polypeptides located in PS II core to PQ. Thereafter, electrons move through PET chain to reach NADP reductase to form energy molecules: NADPH. ATP molecules too form through ATP synthetase using accumulated protons from thylakoid lumen through photolysis of water.

But; in high light condition, when the amount of photons harvested by the chl-protein complexes exceeds the capacity of PET, other energy decay mechanism starts working (Niyogi, 1999). Some part of the EE decays from the pigment-protein complexes via intersystem crossing leading to the production of chl triplets (Chl^3) (Chauvet et al. 1985; Fujimori and Livingston, 1957). In optimal condition, the EE reaches RCs with minimum loss of energy which is often understood as low excitation pressure. But in stressful condition such as suboptimal chilling, the EE decays. There happens spontaneous fractionation of the EE into a few components in PA as well as in RCs. This causes reduction in production of NADPHs and ATPs or reduction in photochemistry (qP or photochemical quenching) and elevation in NPQ (non-photochemical quenching) (Labate et al. 1990; Krall and Edwards 1991; Fryer et al. 1995; Koscielniak and Biesaga-Koscielniak 2006).

Although PET system, photochemical, non-photochemical quenching, precise determination of composition of chl and a variety of carotenoids in maize leaf have been determined and reported in scientific research papers; efficiency of photosynthetic sectors on the basis of RAT, chl and N concentration in central e1 leaf have not been determined in maize to date. The paper includes determination of threshold, upper boundary and optimum range of chl, N conc and RAT measures. This paper includes discovery of a few models to explain photosynthesis through a few models such as RATA Makai, Diversity, NARC and PAY In addition, it also includes discussion of a newly proposed model of 'green car' and 'no car-no food' to explain effects of various proportions of leaf chl and car conc on grain yields. This research also examines significance of frequencies of the e1 leaf sectors of different class range of the SPAD measure.

MATERIALS AND METHODS

Experiment details:

The experiment details have been mentioned in earlier publication (Adhikari et al., 2015a and Sup table 1, 2 & 3). Two RCBD trials of three replications each with newly bred fifteen single cross hybrids of yellow maize were conducted planting on October 03, 2012 in the research field of the NMRP / NARC (National Maize Research Program / Nepal Agriculture Research Council) Rampur, Chitwan, Nepal. Although there were two trials, both trials were adjacent to each other in the same large plot with the same fertilization, manure, sowing patter date and management operations. From the two trials, five pipelines including check hybrids: 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) were examined very closely from the standpoint of photosynthetic leaf sectors of the e1 leaf to extract details of the e1 leaf and grain yield in addition to photosynthetic models. For it, five plants were randomly selected in each of the fifteen plots of the five pipeline hybrids before tassel emergence for minute evaluation of the leaf. Experiment details have been shown in Sup table 1 & 2. Daily temperature data taken from meteorology division of the NMRP / NARC Nepal has been shown in Sup fig 1. Climate of the trial period in the research site is characterized as subtropical; the winter is characterized as suboptimal chilling and minimum / maximum temperatures were -0.30/18.8^{0}C during five days from 95 to 100 DAS (Adhikari et al., 2015b; 2015c) (Sup table 1, 2 & 3).

Red light absorbance-transmittance (RAT) measure, chl and N conc

RAT measures were taken using chlorophyll meter SPAD 502 as SPAD or RAT SPAD measure. The SPAD observations were taken in the region just above the 1.5 cm leaf margin of the leaf in three-day period from 99 to 101 days after sowing (DAS). One hundred measurements of the SPAD were taken from each of the five randomly selected plants from each of the fifteen plots of the five hybrids (Adhikari et al., 2015a). Their leaves were minutely examined through the e1 leaf of the seventy-five row data in Microsoft Excel Worksheet. From the SPAD measure, chl and N conc have been computed using calibrated discovered equations of Cerovic et al., (2012) and Dwyer et al., (1995) for total chl and N contents respectively.

Frequency distribution of e1 leaf sectors and their correlation to grain yields

Characteristics of the FD of the e1 leaf sectors of five hybrids based on varying SPAD measures, chl and N conc have been shown in Sup table 5, Fig 1, 2 & 3 through statistical measure such as mean, trimmed mean, standard deviation, coefficient of variation, minimum, maximum measure, skewness and kurtosis. Pearson's pairwise correlation coefficients (r) have been computed between grain yields and frequencies of the leaf sectors of varying SPAD (Fig 1), chl (Fig 3) and N conc (Fig 2) ranges. From the examination of the r, threshold, upper boundary and optimum range of the SPAD, chl and N conc have been extracted in order to maximize grain yields.

Similarly threshold, upper boundary and optimum range of the RAT, leaf chl and N conc have been determined (Table 1, 2, 3).

RESULTS

Variance analysis and DMRT of frequency distribution of the e1 leaf SPADs
Frequencies of SPAD observations of the e1 leaf of the five pipeline hybrids were examined through variance analysis under four and twenty one SPAD range classes separately. Both tables give identical interpretation, i.e., frequencies of the LS of varying SPAD ra.nge have been found non-significant different among the five hybrids (Sup Table 6 & 7). There is no difference in the non-significance of the frequency of leaf sectors of varying RAT SPAD which inferred through variance analysis (Sup Table 6 & 7).

Threshold (TH), upper boundary (UB) and optimum range (OR) of SPAD in maize leaves
The Pearson's pairwise correlation coefficients (r) between grain yield and frequency of the LS of varying RAT SPAD reflects some information about maize physiologies including photosynthesis. The r reflects about negative or positive and strong or weak effects of the frequencies of the LS of different RAT SPAD range on grain yields. Here; TH, UB and OR of the SPAD have been explored through the r. The TH SPAD is that below which the leaf sectors start demonstrating decline in r to neutral to negative contribution to grain yield; and above which leaf sectors start boosting grain yield. In addition, strong negative r has been found between grain yields and the frequencies of the leaf sectors in different SPAD ranges such as 10-20, 20-30, 30-40, 35-40, 40-45, 0-40, 10-40, 20-40, below 25, 30, 35, 40, 45 and some more (Table 1).

But, strong positive r has been found with the frequencies of the LSSR 45-50, 40-60, 45-55, above 35 and above 40. Strong positive r between grain yields and the LSSR 30-60, 40-60, above 35, 45 added ambiguity to decide perfectly about the TH SPAD. For more precise declaration of the TH SPAD (30, 35, 40, 45) from the perspective of quantitative analysis and subjective judgment; LSSR (leaf sectors in the SPAD range) 30-40 displayed negative correlation with grain yields (Table 1).

We watched minutely and closely on contribution of medium diversity of LS fractionating LSSR 42-54, strong positive r has been obtained with the LSSR 48-50 (0.820), then 50-52 (0.662) and then 46-48 (0.654). From standpoint of the strong positive r too, SPAD 46 is the threshold below which leaf sectors start performing poorly. Leaves with SPAD range 46-52 performs best for grain yield increase in maize. In more accurate and precise interpretation, OR of SPAD will be more appropriate than the TH and UB of SPAD (Table 1; Fig 1).

RATA Makai Model (Red light absorbance-transmittance through antenna)
The RATA Makai model has been proposed from the data taken through RAT of red photons of 650 nm wave length emitted by the SPAD 502. Based on the absorbance of the red photon emitted by the red SPAD diode and migration of the EE to RCs, leaf antenna region has been classified into F RATA Makai, S RATA Makai and L RATA Makai. And first characters F, S and L stand for functional, semi-functional and least functional respectively. So, broad classification of the antenna region chl in the thylakoid membrane in the leaf mesophyll cell chloroplast is F RATA, S RATA and L RATA Makai antenna in the thylakoid membrane in the leaf mesophyll cell chloroplast. Makai is the name of the crop of the maize in Nepalese. The model has emerged from the plant species of the maize from the Nepalese scientists. So, the

Makai has been used instead of maize in the model (Table 1; Fig 1). Grain yield and some phenological data have been shown in (Sup table 4).

Hybrid 6 and 112 were the highest grain yielding and the antenna pigments in the hybrids are of very high functionality to harvest red photons of 650 nm wavelength. Antenna region in the hybrid can be considered as of functional strength (F RATA Makai). But, hybrids 14 and 113 had antenna region of semi-functional strength to absorb the red light (S RATA). But, the hybrid 109 had the antenna region of least functional (L RATA). This way RATA Makai model explains a variety of functionality of the antenna region from the standpoint of their grain yielding potentiality. This can be applied for dry matter production more precisely although we have not reported from the perspective (Table 1; Fig 1).

Furthermore, antenna is consist of pigment-protein complexes such as chl a, chl b and carotenoids. The antenna chl complexes can be broadly classified into three types with their potentialities of red light absorbance-transmittance and transfer of EE to RCs and its effects on grain yields. Highly functional antenna chl-protein complexes harvested red photons of 650 nm and transferred the EE to RCs very efficiently. Antenna in the LSSR 46-52 is in this class based on strong positive r between grain yields and FLSSR46-52. So, it is the F RATA Makai antenna. Antenna in the LSSR 44-46, 52-54, 40-45 and some more sectors which demonstrated weak positive to weak negative correlation is of semi functional antenna region that harvested red photons and transferred the EE to RCs and PET chain with semi-functional strength to contribute to grain yield increase. So, the antenna is S RATA Makai. Antenna in the LSSR 0-40 and some more sectors harvested red photons of 650 nm and demonstrated strong negative r between grain yield and FLSSR 0-40 can be declared as least functional PA region pigment or L RATA Makai (Table 1; Fig 1).

Table 1: Correlation coefficient (r) of grain yield with frequencies of the leaf sectors of varying RAT SPAD measures

SPAD	r	r	SPAD	r	r	SPAD	r	SPAD	r	SPAD	r
0-10	-0.526	-0.530	0-40	-0.697	-0.704	30-50	0.017	35-40	-0.468	Abo 45	0.464
10-20	-0.036	-0.040	0-50	-0.090	-0.090	30-60	0.750	35-45	-0.338	Abo 55	-0.209
20-30	-0.517	-0.517	0-50	-0.090	-0.090	Abo 20	0.222	45-55	0.603	Abo 15	0.451
30-40	-0.496	-0.500	0-60	0.159	0.158	[A]Abo 30	0.441	50-55	0.297	Abo 25	0.272
40-45	-0.266	-0.266	10-30	-0.360	-0.362	Abo 40	0.697	50-65	0.09	Abo 35	0.630
40-50	0.344	0.339	10-40	-0.710	-0.717	Abo 50	0.090	0-25	-0.272	42-44	-0.333
45-50	0.706	0.706	10-50	-0.065	-0.067	Abo 60	-0.159	0-35	-0.630	44-46	-0.170
40-60	0.756	0.744	10-60	0.264	0.264	0-15	-0.451	0-45	-0.464	46-48	0.654
50-60	0.154	0.153	20-50	-0.055	-0.055	5-15	-0.413	0-55	0.209	48-50	0.812
0-20	-0.222	-0.220	20-60	0.189	0.187	15-25	-0.076	5-25	-0.254	50-52	0.661
0-30	-0.441	-0.440	20-40	-0.636	-0.641	25-35	-0.710	25-45	-0.404	52-54	-0.149
N =	1500	500	n =	1500	500	n =	1500	35-55	0.386	n =	1500

[A]Abo is abbreviation for 'above'.

The antenna pigment complexes can be broadly classified into three types with their potentialities of red light absorbance-transmittance and transfer of the EE to RCs: highly functional PA pigments harvest light energy and transfer them to RCs very efficiently; two highest grain yielding hybrids had such antenna. Semi functional antenna region harvest light energy and transfer them with semi-functional strength; two medium grain yielding hybrids had this kind of antenna. And, least functional antenna region chl harvest light energy and transfer them to RCs least efficiently.

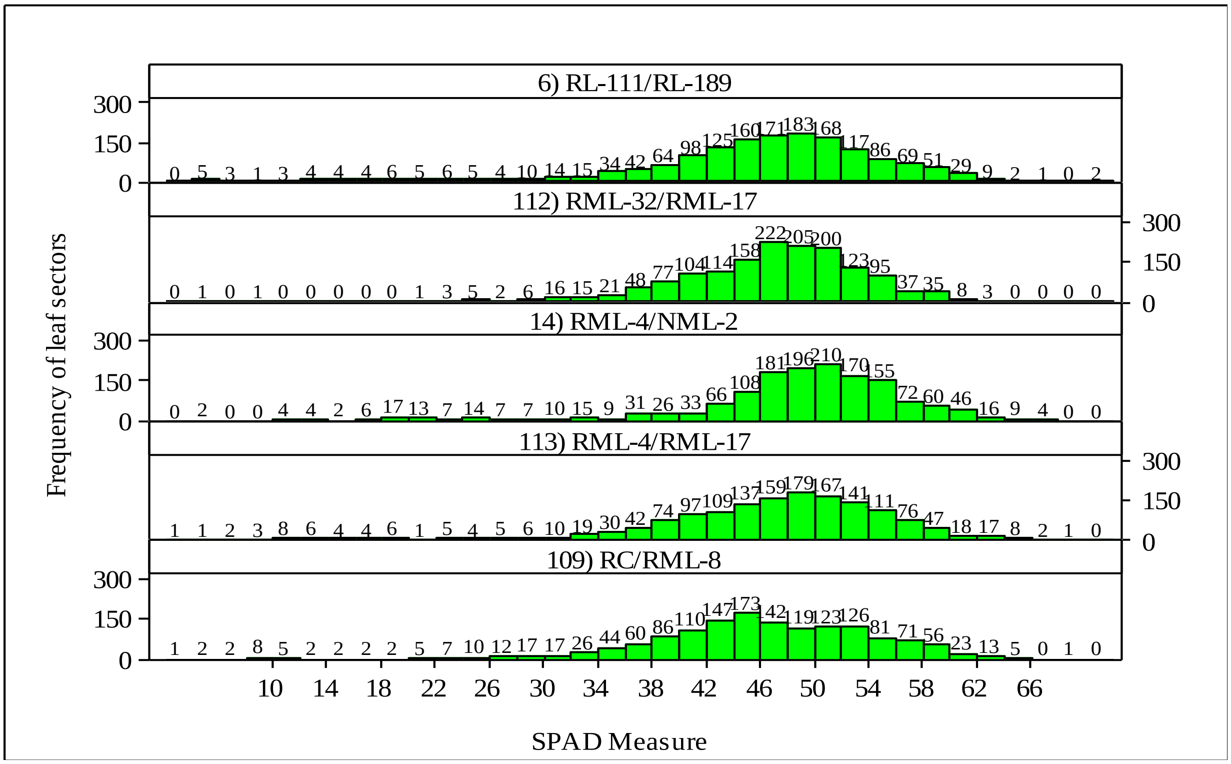

Figure 1: Frequency distribution of e1 leaf sectors based on varying RAT SPAD measures. The Frequency distributions were plotted taking 1500 observations on the fifteen e1 leaves from each of five maize hybrids including recently released Rampur hybrid 2 (RML 4/NML2). The SPAD was recorded in three days continuously from 99 to 101 days after sowing. This figure has been used for different purpose by same authors of the paper (Adhikari, 2015a).

This way RATA Makai model is issued to discuss the efficiency of absorbance-transmittance of red photon by PA chl in presence of the leaf tissue warming infra-red light of 940 nm emitted by the SPAD 502. Higher RAT SPAD measure of the e1 leaf is also meant for increased chl content (Netto et al., 2002; 2005; Cerovic et al., 2012; Escobar-Gutierrez and Combe, 2012). This way RATA Makai model is issued to discuss the efficiency of RAT and migration of the EE by light harvesting PA chl to RCs in presence of the leaf tissue warming infra-red light of 940 nm emitted by the SPAD 502.

Diversity Model

Diversity model explains that there are different types of diversity of the leaf sectors from the standpoint of RAT, chl, N contents, their effects on photosynthesis and grain yield. Based on r, a narrow diversity of LS of narrow SPAD range contributes considerably to grain yield; for instance, medium diversity of the LS that display strong positive r are the LSSP 46-52. The medium diversity of the LS contributes considerably to grain yield; so they are functional diversity. Although broad diversity of LS of broad SPAD range contribute considerably to grain yield, multiplicative nature of certain medium range SPAD diversity lies in the broad range and its major fraction in broad frequency causes such strong positive r. All these diversities of the LS can be declared as functional diversities Besides, the semi-functional and least functional narrow, medium and wide diversity leaf sectors exist in leaves to contribute differentially to grain yields from r between grain yields and frequencies of LS of varying SPAD, chl and N conc (Table 1, 2 & 3; Fig 1, 2 & 3).

Threshold, upper limit and optimum range of chl conc in maize leaves

According to the equation of Cerovic et al. (2012); the SPAD measure of 45 coincides to the total chl conc at 45 µg cm^{-2} on green leaves, the chl conc grows less proportionately in curvilinear pattern below SPAD 45; but grows exponentially and proportionately as the SPAD grows above 45. Correlation analysis of FD graph from the standpoint of chl measure gave very valuable information about required optimum level of chl conc for high photochemical quenching and grain yield. When r between grain yields and the LS of varying chl range of the five pipeline hybrids have been examined, a variety of the LS that displayed strong positive correlation has been observed. So, very minute examination must be done to determine the threshold, upper boundary and optimum chl range in the LS of the maize. Alike optimum range of SPAD, optimum chl conc can be between 46 to 56 µg cm^{-2} (Table 3; Fig 3).

Threshold (TH), upper limit (UB) and optimum range (OR) of N conc in maize leaves

Although strong positive r has been observed in leaf sectors of a variety of N range; subjectively, the OR, TH and UB of leaf N conc should be determined from minute narrow range of N conc in the leaf sectors. From the minute examination of the r between grain yields and frequencies of LS of varying N conc, OR of leaf N has been determined from the strong positive r between grain yield and FLSNR, it is 2.0-2.3% of dry weight. Leaf N conc 2.0% of dry weight has been found TH level for high grain yield and 2.3% N is the UB (Table 2 & Fig 2).

NARC Model (Nitrogen in Antenna and Reaction Centers Model)

Precisely, the name of the model NARC (Nitrogen in Antenna and Reaction Centers Model) has been given to explain how low and high concentration of N in Antenna and Reaction Centers in the leaf mesophyll cells affect on grain yield. In NARC model; amount of leaf N conc is meant for amount of antenna chl-protein complexes, RC located polypeptides, proteins and enzymes in mesophyll cells. This model has been proposed to acknowledge all the scientists and staffs of NMRP/ NARC, Rampur, Chitwan, Nepal who worked in development and maintenance of inbred lines for hybrid maize breeding. As mentioned earlier, N is one of the principal constituents of the leaf chl, protein and enzymes based on works published earlier (Table 2 & Fig 2).

In order to explain the NARC model, r between grain yields and the frequency of leaf sectors of varying N conc have been examined (Table 4, Figure 3). The leaf sectors of N range 2.0-2.3 (2-2.1, 2.1-2.2, 2.2-2.3) displayed strong positive correlation. It implies that light harvesting antenna and RC chl, polypeptides, protein complexes are in optimum conc and activity in the N range. Subsequently, PA chl-protein complexes harvest light energy considerably, transfer the EE to RCs efficiently and there was minimum excitation pressure in the RCs. In addition; it is not necessary that large concentration of N in the LS is involved to high grain yield. For instance, leaf sectors of the N range 2.3-2.7% did not contribute positively and strongly to grain yields (Table 2). As mentioned earlier, PA chl-protein complexes and RC polypeptides in the leaf sector in optimum N range causes increased activity of light reaction in the ARCs (antenna and reaction centers). Higher RAT SPAD measure is generally meant for higher chl and N conc. Very high level of leaf N does not necessarily mean higher proportion of functional RCs per unit leaf area. Below TH N conc, cell physiology is not optimum, the cells are not so multiplicative to make their identity during peak grain filling. Above the UB of N conc, cell physiology is not optimum, the cells are not so multiplicative to make their identity during peak grain filling; or chl, N conc and amounts of soluble proteins are not in parallel growth pattern. In addition, there can be higher proportion of nonfunctional RCs in N conc below TH or above UB. This way, there might be increase of excitation pressure in the antenna and constitutive decay of the EE in RCs in the photosynthetic LSs which lead subsequent reduction in photosynthetic yield causing consequent reduction in grain yield (Table 2 & Fig 2).

It has been reported that N conc is positively correlated to increased efficiency of harvesting of light energy in the antenna region, migration of the excitation energy from antenna region to RCs and transport of electrons from PS II RC II $P680^+$ towards downstream electron acceptors after photolysis of water (Dujardyn and Foyer, 1989; Cen and Sage, 2005; Sharkey, 2005; Jiang et al., 2009; Lin et al., 2009; Yamori et al., 2010).

PAY Model (Model of Proteins amounts and their effects on yield)

Here, the abbreviated word 'PAY' is meant for protein amounts and their effects on yields. In other words, PAY implies amount of leaf proteins, enzymes and their effects on grain yield. Since leaf N is the principal constituent of RUBISCO, Calvin cycle enzymes (Ariovich and Cresswell. 1983; Evans and Terashima, 1987; Lawlor et al., 1987; Khamis et al., 1990; Sage and Pearcy, 1987; Sugiharto et al., 1990; Terashima and Evans, 1988). In addition, all enzymes are proteins. In most laboratories, leaf N is estimated through proteinaceous substance of the leaves. So, leaf N is also proportional to the leaf proteins and enzymes. The PAY model has been extracted from the r between the grain yields and the frequencies of the LS of the five advanced maize hybrids of varying N conc. From logical and subjective analysis of r between LSNR and grain yields, the PAY model has been proposed. The model says neither low, now very high; but, only optimum amount of leaf proteins is positively and strongly correlated to grain yield (Table 4, Fig 3). In the LSNR 2.0-2.3, the amount of the proteins and enzymes enhanced CO_2 fixation and caused consequent increase in grain yield; but LSNR 2.3-2.7 (2.3-2.4, 2.4-2.5, 2.5-2.6, 2.6-2.7) could not contribute considerably to grain yield (Table 2 & Fig 2).

Increase of N conc can be proportional to increase of chl, RUBISCO, PEPC and PODK. In leaves of C4 crop species, RUBISCO is 30% of the total soluble proteins (Sugiyama et al., 1984) and 5–9% of total leaf N (Sage et al., 1987; Makino et al., 2003). This also verifies that N containing all biochemicals including all enzymes are proportional to leaf N concentration. Some Enzymes or soluble proteins are NADP reductase, ATP synthase, RUBISCO in the mesophyll cells and PEPC, PODK, Calvin cycle enzymes in bundle sheath cells. Amount of CO_2 fixation is proportional to the amounts soluble proteins (Martínez et al., 2008; Wingler et al., 2006). It is believed that N conc is also proportional to amounts of cytochrome b6f complex, ferrodoxin, NADP reductase, PS I, NADPH and ATP in the thylakoid membrane and fixation of CO_2 into four carbon compounds in mesophyll cells and production of glyceraldehyde, glucose, sucrose and their derivatives in bundle sheath cells (Dujardyn and Foyer, 1989; Cen and Sage, 2005; Sharkey, 2005; Jiang et al., 2009; Lin et al., 2009; Yamori et al., 2010).

NARC is inorganic whereas PAY is organic model. In NARC, water is photo-oxidized to break down into electron, proton and inorganic oxygen. And electrons and protons are utilized to synthesize energy molecules: NADPH and ATP. But; in PAY model, bundle sheath cells manufacture 3-phosphoglyceradehyde and sucrose utilizing CO_2 and ARC-PET chain-generated energy molecules: ATPs and NADPHs.

Table 2: Correlation coefficient (r) between grain yield and frequencies of the leaf sectors of the ranges of percent dry weight of N content (n=500)

Range	r	Range	r	Range	r	Range	r	Range	r
0-0.75	-0.857	1.5-2	-0.265	1.5-2.0	-0.265	2.0-2.5	0.490	1.8-1.9	-0.398
0.75-1	-0.347	2-2.5	0.490	1.5-2.25	0.247	2.0-2.25	0.692	1.9-2.0	0.288
1-1.25	-0.127	2.5-3	-0.241	1.5-2.5	0.403	0.75-1.25	-0.252	2.0-2.1	0.680
1.25-1.5	-0.632	1-2	-0.356	1.5-2.75	0.536	0.75-1.5	-0.578	2.1-2.2	0.585
1.5-1.75	-0.439	2-3	0.387	1.5-3.0	0.569	0.75-1.75	-0.649	2.2-2.3	0.755
1.75-2	-0.117	Below 2.0	-0.382	1.5-3.25	0.567	0.75-2.0	-0.38	2.3-2.4	-0.218
2-2.25	0.692	Above 2.0	0.382	1.75-3.25	0.642	0.75-2.25	0.124	2.4-2.5	0.053
2.25-2.5	-0.033	1.25-1.75	-0.497	1.75-3.0	0.659	0.75-2.5	0.248	2.5-2.6	-0.352
2.5-2.75	-0.151	2.25-2.75	-0.072	1.75-2.75	0.769	0.75-2.75	0.365	2.6-2.7	-0.134
2.75-3	-0.36	2.25-3.25	-0.125	1.75-2.5	0.748	0.75-3.0	0.329	2.0-2.3	0.696
3-3.25	-0.396	2.0-3.25	0.377	1.75-2.25	0.658	1.0-3.0	0.349	0.75-3.25	-0.214
3.25-3.5	0.647	2.0-2.75	0.452	1.75-2.0	-0.117	1.6-1.7	-0.366		
1-1.5	-0.568	2.75-3.25	-0.369	2-2.75	0.452	1.7-1.8	-0.17		

DISCUSSION

Photochemistry based on SPAD analysis

Below the TH and above the UB of SPAD, it can be inferred that NPQ dominates to qP. But, qP dominates to NPQ in the LSSR 46-52 in maize leaves (Table 1, Fig 1). In the LSSR 46-52, there were optimum chl-protein complexes that absorbed highest light energy, highest amount of the excitation energy migrates to RCs, and the LS had the largest proportion of functional RCs. There was minimum loss of excitation energy (minimum excitation pressure) in RCs through non-photochemical constitutive thermal energy loss in the RCs, efficient PQ pool and downstream electron acceptors-donors. These all caused high PET rate, and high quantum yield for consequent increased grain yield. The LS in the optimum SPAD range implies high activities of ATP synthase, NADP reductase, minimum level of constitutive NPQ through the RCs, the RC polypeptides work effectively in optimal growing condition.

Diversity of leaf sectors (LS)

Maize breeders should consider the diversity of the photosynthetic sectors for leaf trait. Under the character of diversity of LS, the trait of narrow diversity with functional leaf sectors should be the first preferred trait. Then medium diversity with functional leaf sectors should be the second preferred trait. Then the broad diversity of the LS with functional contribution to the grain yield should be third preferred priority trait. Next priority of the character of the diversity of the LS should be the leaf trait of broad diversity with no or least bad sectors. Although; the narrow diversities of LS with high functionality have been preferred best in leaves of our crop cultivar, LS with varying SPAD range may have varying tolerance to insect-pest and disease pathogen. So, broad diversity with optimum SPAD range and with least frequency of very bad

LS should get top priority in selection from parallel grain yielding advanced lines.

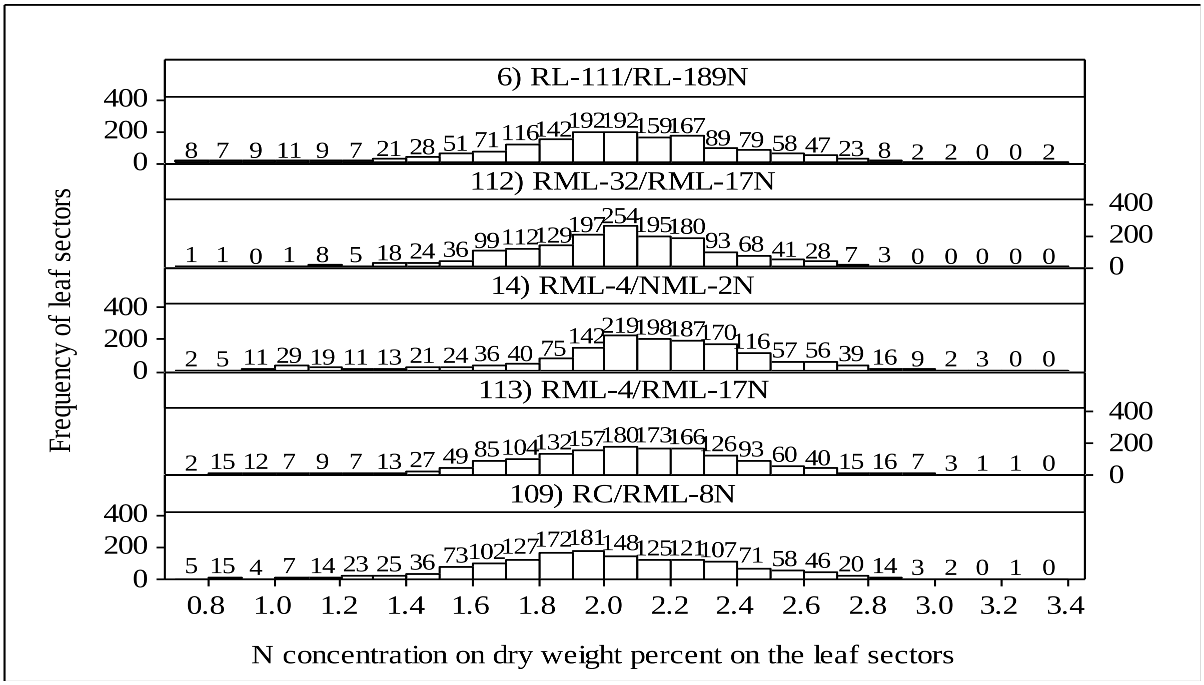

Figure 2: Frequency distribution of e1 leaf sectors based on varying N concentration. The histograms were plotted taking 1500 observations on the fifteen e1 leaves for each of five maize hybrids in three replications under RCBD.

Optimum range of chl conc in maize leaves

In the optimum chl level, the antenna chl can transfer EE to the closest to nearby RCs. PS II $P680^+$ can oxidize enough water molecules dissociating electron, proton and oxygen; The LS in optimum chl range will have optimum proportion of functional RCs, the RCs can eject enough electrons; PQ pool, cytochrome b6f complex and other thylakoid embedded electron acceptors-donors function in higher rate through efficient redox reactions; NADP reductase and ATP synthase too can produce enough energy molecules for CO_2 fixation. This way, the qP dominates NPQ in such LS (Table 9). In precise interpretation, chl 46 µg cm^{-2} has been declared as the threshold below which LS cannot contribute to grain yield positively. In the LSCHLR 46-56 µg cm^{-2}, chl can harvest enough red photons of 650 nm wave length. But in LS with chl conc below 46 µg cm^{-2}, antenna chl conc could not harvest enough red photons of 650 nm; transfer enough excitation energy to the closest RCs; low concentration of $P680^+$ could not oxidize enough water into protons, electrons and oxygen; transfer excited electrons to PQ pool. The consequences impaired PET chain efficiency and caused subsequent heavy reduction in the production of ATPs and NADPs. Chl conc above 56 µg cm^{-2} could not demonstrate positive r to grain yield. In addition, mesophyll cells with chl conc above 56 µg cm^{-2} could not be multiplicative, highly functional to contribute to grain yield. Instead, unparalleled growth of ch and car conc cannot protect photosynthetic apparatus and can damage the apparatuses through photo oxidation. So, the chl conc above 56 µg cm^{-2} can be more than waste.

Chlorophyll conc in suboptimal temperature range
Leaves of maize plants developed in chilling temperature range had lower quantum yield of electron transfer from PSII (Fv/Fm) to downstream electron carriers and lower quantum efficiency of CO_2 fixation (ϕCO_2) than leaves of the plant developed in optimum temperature in natural growing conditions (Andrews et al 1995; Fryer et al., 1998; Leipner et al., 1999; Ying et al., 2002). As the temperature goes down from optimum ($30/25^0$C) to suboptimum temperature ($14/12^0$C), development of photosynthetic apparatus becomes poorer which is indicated by declined chlorophyll content per unit leaf area, reduced physiology of PS I and PS II and lower CO_2 assimilation (Nie and Baker, 1991).

Green Car Model
Here, green is for chl and cars are for carotenoids. Green and car pigments are coordinated in maize leaves for optimum photosynthesis as 'green car' gives message of coordination. We have observed that FLSCHLR below 46 µg cm^{-2} and above 56 µg cm^{-2} demonstrated negative r. Very low and very high chl conc could not demonstrate positive r (Table 3 & Fig 3). This information triggered the mind to develop a model to demonstrate effects of different proportions of chl conc. Results of Toth et al. (2002) and Haldiman (1999) also strengthened the mind to propose a model of 'green car' for the effects of different proportion of green and cars in leaf mesophyll cells on grain yield. This model explains about the coordination between the green and car pigments, effects of low or high or optimum range of green and cars in maize leaf sectors in light reaction components, photochemistry and grain yield. From minute analysis of data and figure of the trend of chl and cars in response to N fertilization (Toth et al., (2002) and r between grain yield and LSCHLR in the present study; we proposed "Green car model".

For harvest of light energy by the leaf, there must be enough chl conc. Elevated level of chl can harvest enough light energy. But; under suboptimal temperature in winter chilling, even low sunshine detrimentally injures the photosynthetic apparatus. Besides; as leaf chl level increases, car level does not rise in parallel after a certain range of chl conc. In addition, low cars cannot protect conformation of chl-protein complexes from sunlight radiance. Subsequently, it damages photosynthetic apparatuses. Consequently, less light energy is harvested by increased level of chl and the EE cannot migrate from injured chl-protein complexes to RCs efficiently. Then the excess EE must be transferred from chl triplet to car through Dexter exchange mechanism (Siefermann-Harms, 1987) and dissipated from the car triplet in thermal form. But, the low car concentration associated xanthophyll cycle pool dissipates excess excitation energy least efficiently. Subsequently, the radiation damages photosynthetic apparatus by photo oxidation. The EE cannot migrate from the antenna chl to the closest RCs of LSCHLR 55-65 and above. In addition, RCs and PQ pool are not so efficient for charge separation. Strong positive effect of the functional LSCHLR on grain yields of the maize hybrids and amount and proportion of cars and chl in the LS in response to amount of N fertilization (Toth et al., 2002) help illustrate green car model (Table 3, Fig 3).

In other words, low chl is meant for poor harvest of light energy, low photochemical quenching, poor transport of electrons from PS II to downstream cytochrome b6f complex, ferrodoxin, NADP reductase, ATP synthase, PS I, formation of energy molecules: NADPH and ATP (Holt et al., 1993; El-Lithy et al., 2005). In addition, cars work depletion of singlet oxygen (Koyama, 1991) as well as quenching of triplet of the chl^3 (Scho¨del et al., 1999; Farmilo and Wilkinson, 1973; Land et al., 1971) through Dexter Exchange Mechanism forming car triplet (Siefermann-Harms, 1987) and then car^3 dissipate excess excitation energy. This NPQ protects photosynthetic apparatus from photo-oxidative damage. Stay-efficient photosynthetic apparatus helps maintain proportion of the functional RCs and enhance efficiency of PQ pool to accept electrons from the RCs in the LSCHLR 46-56 (Table 3& Fig 3).

Thus, green and car pigments work in coordination of associated protein complexes between them. In essence; strong positive effect of high green-high cars on high grain yield; But, weak positive effects low green-low car to cause low grain yield; high green-low car to cause low grain yield and low green-high car on low grain yield became conspicuous. Optimum level of cars with optimum level of chl goes together for optimum grain yield. Similarly increased level of chl and proportionate increased level of car goes together. This way, the Green Car Model has been proposed to explain effects of proportion of green and cars on component physiologies of light reaction in maize leaves as well as it effects in grain yield. Effect of low car can also be explained in next model proposing “low car-low food model” or “no car-no food model” (Table 3, Fig 3).

Leaf carotenoid complexes in PA around PS II core protect light-harvesting complexes against singlet-oxygen formation (Koyama, 1991) by two mechanisms: quenching chl triplets (Schodel et al., 1999) receiving the excited energy of chl complex and direct scavenging of the singlet oxygen (Farmilo and Wilkinson, 1973). For it, carotenoids (cars) receive triplets of chl^3 and form car triplets (car^3) and the car^3 decays the excited energy and comes to ground state dissipating heat (Land et al., 1971). For the transfer of energy, fluorescence quenching denominated as Dexter exchange mechanism (Siefermann-Harms, 1987) takes place to transfer the triplet from chl^3 to car. For it, tightly bound protein complexes coordinate between chl^3 and cars (Siefermann-Harms, 1987). This way; fast energy transfer from chl^3 to car and then thermal energy dissipation from car^3 takes place for efficient photo-protection of the photosynthetic apparatus.

Table 3: Correlation coefficient (r) of grain yield with frequencies of the leaf sectors of varying ranges of chlorophyll

CHL	r	CHL	r	CHL	r	CHL	r	CHL	r
0-10	-0.574	0-40	-0.590	50-65	0.396	20-60	0.166	5-55	0.061
10-20	0.018	0-50	-0.248	0-25	-0.465	20-40	-0.489	15-35	-0.746
20-30	-0.728	0-60	0.149	0-35	-0.741	30-50	-0.115	15-45	-0.436
30-40	-0.392	0-15	-0.355	0-45	-0.47	30-60	0.291	15-55	0.106
40-45	-0.293	5-15	-0.448	0-55	0.07	Above 20	0.21	25-45	-0.365
40-50	0.206	15-25	-0.426	5-25	-0.508	Above 30	0.551	35-55	0.348
45-50	0.699	25-35	-0.627	10-30	-0.46	Above 40	0.590	Above 15	0.355
40-60	0.735	35-40	-0.242	10-40	-0.573	Above 50	0.248	Above 25	0.465
50-60	0.48	35-45	-0.276	10-50	-0.215	Above 60	-0.149	Above 35	0.741
0-20	-0.21	45-55	0.734	10-60	0.216	5-35	-0.781	Above 45	0.470
0-30	-0.551	50-55	0.666	20-50	-0.192	5-45	-0.482	Above 55	-0.07
44-46	-0.001	48-50	0.687	52-54	0.496	56-58	-0.145	60-62	0.197
46-48	0.711	50-52	0.735	54-56	0.805	58-60	-0.161		

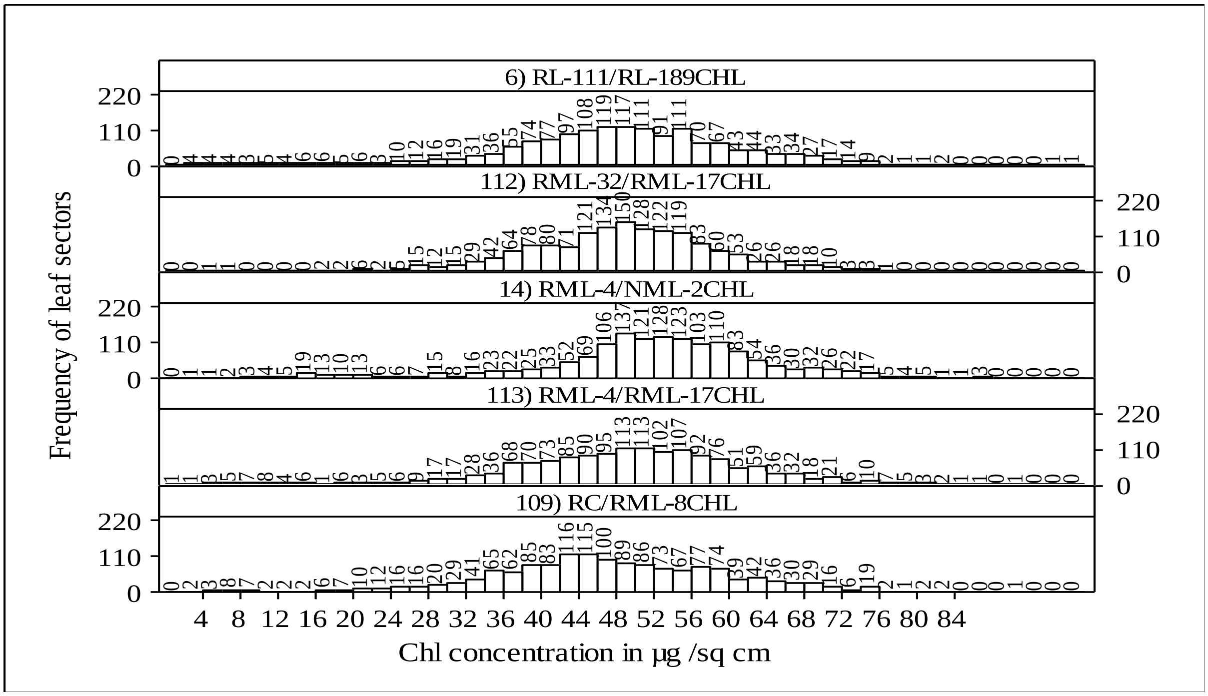

Figure 3: Frequency distribution of e1 leaf sectors based on varying chl conc. The histograms were plotted taking 1500 observations on the fifteen e1 leaves for each of five maize hybrids in three replications under RCBD

Furthermore, excessive accumulation of N in the maize leaves is also understood that the leaf will have less efficient xanthophyll cycle pool to protect photosynthetic apparatuses. The less efficient xanthophyll cycle pool does not let leaf develop protective pigments to protect the green pigment and photosynthetic apparatus (Toth et al., 2002). Vice versa is the case if N is in the optimum range in maize leaves (Toth et al., 2002). Toth et al., (2002) too demonstrated that excessive N and chl in leaves did not cause any considerable and significant increment in grain yield in maize at all (Toth et al., 2002). It is very clear that low N and chl too does not let cultivar produce considerably. For instance, leaf with SPAD 35-45 could not contribute to grain yield considerably (Table 2). Such leaf sectors can be declared having low photosynthetic efficiency from narrow leaf sector perspective. Low photosynthetic LS can have such photosynthetic apparatus such as PQ and cytochrome b6f complex that cannot accept electrons efficiently from PS II. Then PS II is locked or closed since it could not transfer electrons to PET chain. As the result, electron deficiency in PS I could not be fulfilled. This way; photochemical reactions of the RC chl a protein complex of the PS I and PS II could not go ahead. Subsequently, photochemistry output such as generation of energy molecules: NADPHs and ATPs will be less than requirement (Jiang et al., 2009; Lin et al, 2009; Yamori et al., 2010) for enough CO_2 fixation (Genty et al., 1989; Edwards and Baker, 1993).

Khamis et al., (1990) demonstrated leaf nitrate N deficiency increased leaf zeaxanthin content which is responsible to protect the photosynthetic apparatus through thermal dissipation. Low car associated xanthophyll cycle is an abnormality that cannot protect photosynthetic apparatus from photooxidative damages. This causes inefficient NPQ and damages photosynthetic apparatus leading reduction in quantum yield and grain yield. So, low car-low food or no car-no food model has been proposed (Table 3, Fig 3).

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We have done this work in subtropical northern hemisphere in winter and the plants were exposed to natural extreme chilling in evening to morning during flowering, early and middle grain-filling. In addition, the trial crop could tolerate chilling for longer duration. Extreme chilling below 15^0C disturbs phenomena of the photochemistry for maximum quantum yield which has been reflected by Savitch et al. (2009). Furthermore, tolerance to the chilling temperature also varies among the maize hybrids. So Pearson's r could not reflect complex interactions of several component physiologies of the photosynthesis in pairwise linear language between grain yields and frequency of leaf sectors in some N range although N conc was not less than optimum level. The non-significant micro-variation in the e1 leaf region has been found. The variation in the leaf sectors might have developed because of micro-variation in soil bulk density, moisture, nutrient content and orientation of the leaf regions to sunshine every day. The very micro-variation too has been exploited in the research work to find the fruitful and useful scientific information .

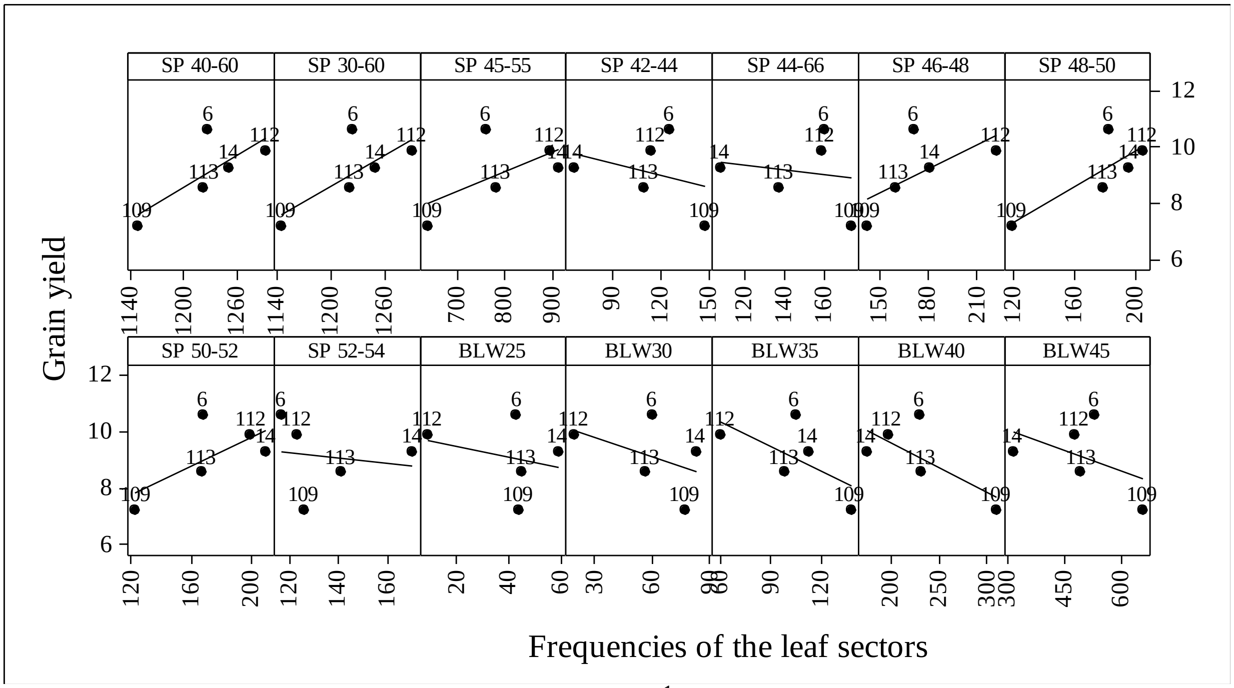

Figure 4: Graphs plotted between grain yields (t ha^{-1}) and frequencies of the e1 leaf sectors of varying SPAD ranges out of 1500 sectors. Here SP 44-46 is meant for the SPAD just above of 44 to 46. Similarly the range is meant. Here, BLW SPAD is abbreviated for 'below SPAD'. Numbers in graph indicate hybrid numbers. Hybrids 6, 112, 14, 113 and 109 are in decreasing order of grain yields.

SPAD 25, 35, 40, 46

Netto et al. (2005) reported that SPAD below 40 indicates impairment of photochemical phenomena such as PET rate in coffee leaves. But; in my research work in this paper; without fluorescence study; but correlating between grain yields and frequencies of the leaf sectors of different SPAD range including narrow diversity says that SPAD 46 is the threshold. But; in order to differentiate leaf sectors into very bad sectors, wide diversity of the leaf sectors have been examined to find out strong positive and strong negative r bearing LS. Accordingly;

subjective and logical analysis of r; frequency of the LSSR (leaf sectors in the SPAD range); SPAD 35 and 25 can be two markers below which photosynthetic leaf sectors can be classified to bad and very bad respectively (Fig 3). Furthermore, SPAD 25 is effective marker than 40 or 46. For instance; the second pipeline hybrid 112 which had lowest frequency of the LS below SPAD 25 on e1 leaf. It has high tolerance to abiotic and biotic stresses. So it can be advanced to the variety release committee (Table 4).

CONCLUSIONS

The highest grain yielding hybrid 6 demonstrated wide TAI and SAI and had larger frequency of very bad LS. These are the indices of aberrations or sensitiveness to biotic and or abiotic stresses. Hybrids 14, 113 and 109 all had larger frequencies of bad LS in comparison to the hybrid 112. Furthermore; the frequencies of the bad LS implies that the hybrids 14, 113 and 109 can fail any time to give harvest since they can be victim of any minor disease pathogen to destroy in unpredictable way and in unpredicted climate change associated prolonged duration of chilling or extreme fall of temperature (Oliver et al., 2005; Solomon et al., 2007; Sataka and Hayase, 1970) in combination with excessive dose of N fertilizers (Hayashi, 2006; Hayashi et al., 2009). One of such examples is the drop of corn yield in the year of 1970 in the corn belt in the USA because of leaf blight (Laughnan and Gabby-Laughnan 1983). It can be discussed that very high SPAD in leaves of the maize cultivar imply that there was waste of N resources since higher SPAD above the upper boundary could not induce for proportionate grain yield increment (LSSR 52-54). Leaf SPAD 52-54 or above it could not contribute to grain yield considerably (Table 4). At the end, such close-up examination of the leaf too can help foretell about the fate of the superior pipelines.

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SUPPLEMENTARY 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 "Discovery of a few photosynthetic models through red light absorbance-transmittance of the e1 leaf sectors of newly bred hybrids of maize (*Zea mays* L.)."

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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