Dynamics of chlorophyll and N concentration on e0 and e3 leaves of maize hybrids in winter in subtropical region

Red light absorbance through maize

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ABSTRACT

National maize productivity is very low in Nepal. Increase of the productivity is only possible through growing high grain yielding single cross hybrid maize cultivar. So, development and evaluation of maize hybrids are principal steps to select high grain yielding and superior hybrid for cultivar. For it, fifteen newly bred single cross hybrids of yellow maize were examined from the standpoint of chlorophyll (chl) dynamics and their effects on grain yield. For it, a trial of RCBD experiment was conducted in open field in winter in subtropical region in Nepal. Seeds were sown on October 3, 2012 plot in two row plot area of 1.4 x 3.0 m^2. After anthesis, chl and N concentration (conc) implying RAT (red light absorbance-transmittance) SPAD measure were taken from the topmost ear (e0) and third (e3) leaf above the e0 in ten days interval during grain filling. SPAD measures were transformed to total chl and N conc. E0 leaf has been found more grain yield determining than e3 leaf and terminal grain filling duration has been found more determining than early grain filling from the

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standpoint of correlation of grain yield with chl, N conc and SPAD measure. From pooled variance analysis; SPAD and chl conc were not significant different in different leaves and among the hybrids (Hybrids x Leaves x Ages). But, the SPAD and chl conc were significant different among the two leaves and ages (Leaves x Ages) irrespective of the hybrids. Different to the SPAD and chl conc, N conc was significant different in the leaves among the hybrids with respect to ages of the plants among the hybrids (Hybrids x Leaves x Ages). Thirteen top high grain yielding hybrids (HGYHs) 8, 12, 11, 13, 5, 6, 10, 1, 7, 14, 2, 9 and 15 were non-significant different from the standpoint of grain yield. The SPADs were in the non-significant range of 51-55 in e0 leaf in the duration from 95 to 125 d among the fifteen hybrids. Among the top four HGYHs 8, 12, 11 and 13; hybrid 11 lost chl and N from e0 leaf significantly on 135th d relative to the most of the hybrids. It means that the hybrid 11 could efficiently degrade N containing soluble protein and chl even from e0 leaf relatively. Top five HGYHS 8, 12, 11, 5 and 6 except 13 among the top listed ten hybrids constantly maintained chl and N conc implying SPAD in the e0 leaf up to the 135th d. In addition, it implies that these five hybrids sent newly up-taken N to kernels without degradation of the proteins and chl from the e0 leaf till the age of 135 d. HGYH 8 had degraded soluble proteins and enzymes and chl in e3 leaf and mobilized the degraded N to the kernels more efficiently from the e3 leaf. It is not necessary that maize hybrids must constantly maintain soluble proteins and chl conc during most of early to mid-grain filling duration for high grain yield. Instead, efficient reasonable decline of the soluble protein and chl conc during early to mid-grain filling also accelerate grain filling phenomena.

INTRODUCTION

N and chl conc in grain producing crop species vary among different leaves, reproductive and whole plant parts during entire phase of growth, development and crop maturity phase. Efficiencies of N uptake by roots from low soil N level (Graham, 1984; Satelmacher et al., 1994) and its utilization (Chevalier and Schrader, 1977; Moll et al., 1982; Perez Leroux and Long, 1994; Presterl et al., (2003) also vary (Balko and Russel, 1980; Muruli and Paulsen, 1981; Lafitte and Edmeades, 1994; Banziger et al., 1997; Bertin and Gallais, 2000) for different physiological phenomena to the formation of different plant parts including reproductive organ to grain production. Maize genotypes too vary in the efficiencies of N uptake and its utilization in the plant system (Ding et al. (2005) for the successes (Presterl et al., 2003) of reproductive and high grain yielding. So, the study of the dynamics of N (Dywer et al., 1995), its derived chl conc (Escobar-Gutierrez and Combe, 2012) and their influence on grain yield production (Ding et al., 2005; Muchow 1994) are very important to select for the highest efficiencies (Graham, 1984; Satelmacher et al., 1994) minimizing its loss through leaching into underground and drained water from the crop field.

Explanation about the importance of chl, proteins and enzymes and N in leaf cells is beyond the scope of the paper. About 50 to 70% N is in different forms in leaf cell chloroplasts (Stocking and Ongun, 1962; Hageman, 1986) and remaining N is in proteins and enzymes. Correct estimation of leaf chl and N conc has become easy through imaging technique. Red light absorbance-transmittance measuring device such as chlorophyll meter ‘SPAD 502’ is handy and its measures imply about conc of leaf chl and N very easily (Minolta, 1989). So the device can be very useful to breeders to crop modelers. The advent of SPAD 502 made possible for easy, precise and non-destructive measurement of the leaf chlo and N conc. The reading obtained by the leaf SPAD-502 device is curvilinearly and exponentially proportional to the leaf chl conc (Escobar-Gutierrez and Combe, 2012; Cerovic et al., 2012). Their equations have made precise transformations of the SPAD into chl conc. The SPAD has been found correlated to leaf N conc and precisely in quadratic-plateu pattern in central leaves of maize (Dwyer et al, 1995). The SPAD measure is mainly influenced by absorbance-transmittance red light of 650 nm by the leaf chl conc (Markwell et al., 1995). In addition, Rubisco alone shares 30%, but Rubisco, PEPC and pyruvate orthophosphate dikinase shares 50% of the total soluble proteins in leaves of C4 plant species (Sugiyama et al., 1984; Sage et al., 1987; Makino et al., 2003). But, most of the hybrids have parallel dynamics of N and chl concentration in the leaves except in long-stay-green genotypes (Thomas et al., 2002; Ho¨rtensteiner and Feller, 2002; Ho¨rtensteiner, 2006).

Although chl, N and xanthophyll pigment components of maize leaves as a whole have been analyzed and reported from the standpoint of applied fertilizers, chilling stress, grain yielding performance and protection of photosynthetic apparatuses in advance level by Haldiman, (1999); Toth et al. (2002); Savitch et al. (2009); chl and N conc of e0 and e3 leaves during entire grain filling duration have not been recorded separately with non-destructive method to date. In order to examine dynamics of chl and N conc in e0 and e3 leaves of newly bred single cross hybrids of yellow maize and their effects on grain yields in winter of the subtropical region in Nepal, the study was performed. The work is to determine whether N, chl conc and SPAD measures in leaves can be exploited for estimating grain yield of newly developed genotypes discovering regression equations and based on R-square. So, plant breeders can use such equations as selection marker in breeding and crop modelers can refine the equations for estimation of grain yield and production in their region through crop model research works of particular cultivars. Precision interpretation about significance of stagnant versus fluctuating chl and N conc in e0 and e3 leaves during grain filling have not been reported in reference to newly developed single cross maize hybrids to date. So, it also includes the aspects.

MATERIAL AND METHODS

Details of the experimental site

The hybrid trial was conducted in the research plot of National Maize Research Program (NMRP) in Nepal Agriculture Research Council (NARC). The location 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.

Treatment details

The hybrids that were included in the trial were developed at the same experimental site as mentioned in Sup Table 1. Gaurav (entry number 15) is a newly released single cross hybrid was also inside the fifteen hybrids as standard check. The plant materials (F_1 generation) seeds obtained in March 2012 for hybrid trial A_1 are shown in Sup Table 1 (S for supplemental).

Crop management in the trial

Sun hemp was grown as green manure crop and ploughed on mid May 2012 before winter hybrid trial. Then organic manure was applied @ 33 t ha^{-1}. The field was finely ploughed and made clod free. Application of chemical fertilizer was done @ 120:60:40 kg N, P_2O_5 and K_2O ha^{-1}. In basal dose, 50% N, all phosphorus and potassium fertilizers was applied in the form of DAP and murate of potash; and 50% N was applied in split dose as top dressing at rate of 30 and 30 kg in the form of urea on 45 and 60 d after sowing (DAS). For the trial, seeds of fifteen newly developed single cross hybrids of yellow maize were planted manually on October 03, 2012. Two seeds were dropped on 0.25 m spacing in each of the two rows in each plot of 3 x 1.4 m^2. Each block of fifteen plots was separated with an alley of 1 m. The row direction was on north-south. Plant population of twenty four plants was maintained in each plot on 30 DAS to keep density 57143 plants ha^{-2}. Soil loosening and weed removal was done manually on 30 DAS and earthing-up was done on 45 DAS. Four furrow irrigations were done on 50, 70, 90 and 110 DAS through shallow tube well of 4” pipe. The crop was harvested on 185th day.

Observations of the data

Five plants were randomly selected in each plot before tassel emergence. The recording of RAT SPAD measure was done from just above of 1.5 cm leaf margin on the topmost ear (E0, or e0) and third leaf (E3 or e3) above e0 using Chlorophyll meter SPAD 502 (MINOLTA, 1989). Data were recorded on 95, 105, 115, 125, 135, 145, 155 DAS. Weather data of Rampur-Chitwan, Nepal of the trial duration has been shown in tabular form (Sup Table 3). For chl estimation, the valid equation of $Y = (99 *X) / (144- X)$ (in $\mu g/cm^2$) has been used (Cerovic et al. (2012) and shown in Sup Table 2. Days for 100% population senescence was recorded for each plot and shown for each hybrid with the denotation of POP SEN100% in Sup Table 5A.

To determine grain yield of each plot, shelling of ears of the each plot was done and bulked kernels were weighed for each plot. Moisture percent of kernels were determined taking a sample of bulked lot of the plot kernels using moisture meter. Yield of each plot of 3 x 1.4 m^2 was transferred to t ha^{-1}. Similarly; grain yield t ha^{-1} was transferred at 15% moisture level using simple arithmetic formula [(100-seed moisture %)*Grain yield]/ (100-required moisture). Data were handled and processed through the spreadsheet of Microsoft Excel 2010; variance analysis and DMRT (Duncan Multiple Range Test) were done through Genestat and MSTAT-C; equations and graphs have been extracted using Minitab.

RESULTS

Growing days of the hybrids in the winter

Ten days GDD (growing degree days) was maximum (144^0days) when the seedlings were growing, the GDD declined below zero (-7.6^0days) during peak grain filling in the winter, then again started rising till the GDD reached max (143^0days) in the last ten days duration of the crop harvest (Sup Table 3).

Variance analysis of leaf chlorophyll, N and SPAD of the hybrids

From pooled analysis of variance; the fifteen hybrids of the yellow maize have been found non-significant different from the standpoint of chl conc and RAT SPAD measure (Hybrids x Leaves x Stages); but, significant different from the standpoint of N contents on e0 and e3 leaves on seven stages (Hybrids x Leaves x Stages) during grain filling in the winter (Table 3). Again; variance analysis of leaf chl content and RAT SPAD on e0 and e3 leaves of the fifteen hybrids was done separately (Table 4). The hybrids (Hybrid x Stages of e0 leaf) have been found uniform or non-significant different in e0 leaf; but significant different on e3 leaf from the standpoint of chl conc, its synthesis and maintenance; uptake, upward movement of nitrogen; maintenance of photosystems, proteins and enzymes such as RUBISCO, PEP carboxylase that contain N from 95 to 155 DAS during grain filling.

From the individual variance analysis; the fifteen hybrids have been found non-significant different from the perspective of chl, N conc and RAT SPAD on e0 leaf during most of the grain filling period except the stage after 135 days after sowing. Chl, N contents and RAT SPAD measure of the e0 leaf of the hybrids were almost uniform from 95 to 135 DAS; but differential synthesis and degradation of chl-protein complexes after 135 days caused significant variation among the hybrids from the standpoint of e0 leaf chl, nitrogen conc and RAT SPAD measure. Again after 145 DAS to 155 days, the hybrids have been found almost uniform through the phenomena of significant differential faster chl degradation, declined enzymes and protein complexes (Table 5A, 5B) (Sup Table 4A, B, C & D; Sup Table 5A, B, C & D).

Evaluation of the hybrids

All the fifteen hybrids had e0 leaf RAT SPAD measure almost uniform statistically among themselves during most of the grain filling till 145th day. Thereafter, sharp decline of the SPAD measure occurred on the e0 leaf. But, from the standpoint of top e3 leaf; those fifteen hybrids pulled N and developed chl-N contents on e3 leaf with almost equal strength among themselves except few hybrids 7 and 15 during early grain filling (ten days earlier to 95th d). Thereafter, significant differentiation of chl and N conc started to occur on the e3 leaf among the fifteen hybrids (Table 5A, 5B); (Sup Table 5A, B, C, D).

According to Dwyer et al (1995), N decline happens on central leaf immediately after flowering. Hybrid 8 might have continued high N uptake efficiency even in post-flowering late grain filling duration; so it did not let chl conc fall on the e0 leaf sharply (opposite to the findings of Below, 1997). Low level of RAT SPAD on the e3 leaf of the hybrid 8 indicates that its kernels had stronger potentiality and higher strength to attract N associated assimilates towards ear for its development to yield higher than synthesis and maintenance of chl conc on the e3 leaf.

Among the newly bred single cross hybrids; more hybrids might have more than one weakness for not selecting them for advancement and release. So; a single reason cannot be enough scientific logic to explain the reasons of low grain yield for a particular single cross hybrid. Among medium GYHs 10, 1, 7, 14, 2 and 9; H 10 had SPAD, chl and N values highest on e0 leaf in 95 to 155 DAS. The RAT SPAD and its derived N and Chl conc whether they are above or below the threshold have been found semi-functional for grain yield contribution based on the grain yielding potentiality (Table 5A).

Medium grain yielding hybrids 10, 1, 7, 14, 2, 9 and 15 had developed top leaves during the start of winter chilling; so, they might have less efficiently developed photosynthetic apparatus in the light intensity and low temperature which can be concluded from the findings of researches done (Baker et al., 1994; Haldiman, 1999; Jompuk, 2004). The top leaves that develop in the natural winter chilling may have lower photosynthetic capacity, lower quantum efficiency of CO_2 fixation (ΦCO_2) and lower quantum efficiency of PET at PS II (Φ_{PSII}) than juvenile leaves which develop in favorable terminal autumn duration which can be inferred from the experiments of Nie et al., 1992; Fryer et al., 1998; Leipner et al., 1999. High intensity light and suboptimal chilling temperature can cause inhibition of photosynthesis (Farage and Long, 1987). Another most important reason can be inbreds used for the hybrid development might not be of distance genetic diversity. So the hybrids 10, 1, 7, 14, 2, 9 and 15 might have been found medium grain yielding.

In low grain yielding hybrids 15, 4 and 3; high, or medium, or threshold or lower than threshold or almost inside the optimum range of chl, N and SPAD range on e0 and e3 leaves have been found similar to the highest grain yielding hybrids during the grain filling. But the Hs 15, 4 and 3 were low grain yielder. The N and chl conc among the low grain yielding hybrids have been declared as least functional since the photosynthetic efficiency could not contribute well to grain yield (Table 5A, 5B; Fig 1, 2). It will be earlier to explain about the reason of poor contribution of chl and N containing proteins and enzymes of the leaves to the grain yield. Low efficiency of mobilization of photo-assimilates to the kernels, phloem loading-unloading, high callose deposition in sieve tube elements, least active stem reserve mobilization, low proportion of functional RCs, high constitutive NPQ, high antenna NPQ, poor conformation of pigment-protein complexes during winter chilling can be some of the physiological reasons of the low yielders.

Dynamics of SPAD, chl and N conc on e0 and e3 leaves

Growth and decline SPAD measure on e0 and e3 leaves with response to the age of the plant can be easily reflected through the discovered regression equations with very high coefficient of determination ‘R-square’. It reflects dynamics of chl and N conc in maize leaves. In the equation; Y is SPAD measure and X is days after sowing of the crop plants during grain filling (Eq 1 and 2, Fig 1; Table 5A, B) (Sup Table 5A, B, C & D).

The following are pooled equations of chl and N dynamics for all the fifteen hybrids.

$Y = 546.0 - 12.35\,X + 0.1023\,X^2 - 0.000281\,X^3$, R-Sq = 93.7% (e0 leaf) Eq 1

$Y = 409.1 - 9.195\,X + 0.\,0774X^2 - 0.000214\,X^{3,}$ R-Sq = 98.4% (e3 leaf) Eq 2

[Where Y is SPAD value on e0 and e3 leaves and X is numbers of days after sowing (DAS).]

Correlation coefficient (r) of grain yield with leaf SPAD measure

Grain yields have been found positively and strongly correlated to the SPAD measure on e0 than e3 leaf among the fifteen hybrids. Besides, highest correlation was observed on 135 and 145 DAS than early grain filling. Although the leaf chl and N conc were higher in early grain filling duration (earlier to 135 DAS); SPAD measure could not display strong positive correlation. So, grain yield estimating equations discovered from the SPAD measure did not have not high R-square (Eqs 3 to 8) (Table 6A, & B). There is a weak or poor source-sink relation among the newly bred single cross hybrids of maize since not so strong positive r has been found between grain yield and chl-N content and SPAD measure of e0 and e3 leaf among the fifteen. The r of about 0.50 between grain yield and e0 chl on 145 DAS (Fig 2C) implies that 8 to 9 hybrids have strong source-sink relation at the stage, but some hybrids did not strongly followed the strong positive correlating nature with grain yield. But, hybrid 13 deviated from the positive correlation pattern. So, the single hybrid 13 lowered the r (Fig 2A-C).

Grain yield in t /ha (Y) estimating equation from leaf greenness SPAD measure

$Y = 24.47 - 0.8187\ E3SP145 + 0.01104\ E3SP145^2$, R-Sq = 22.3% --Eq 3
$Y = 4.68 + 0.123\ E3SP145$, R-Sq = 15.9% --Eq 4
$Y = 290.1 - 11.23\ E0SP135 + 0.1124\ E0SP135^2$, R-Sq = 33.9% --Eq 5
$Y = - 2.248 + 0.2421\ E0SP135$, R-Sq = 13.3% --Eq 6
$Y = 62.60 - 2.284\ E0SP145 + 0.02450\ E0SP145^2$, R-Sq = 24.4% --Eq 7
$Y = - 2.387 + 0.2431\ E0SP145$, R-Sq = 21.9% --Eq 8

Genetic system of the hybrids for the strength to utilize leaf N and chl conc

Genetic system of the HGYHs can be said of strong strength to utilize N containing soluble proteins and chl conc in their leaves in winter in the subtropical region. A variety of HGYHs can be identified from the standpoint of stagnant high versus low or declining chl and N conc on the e0 and e3 leaves. Hybrid 13 maintained lowest chl and N conc in the e0 leaf. The hybrid 13 had chl and N conc in the e3 leaf was low, but not so less than HGYH 8. H8 had high chl and N conc in e0 leaf but low conc in e3. But, it was the highest yielder among the top five HGYHs (Fig 1, Table 5A). Medium to low grain yielding hybrids 2, 9, 15, 4 and 3 respectively had chl and N conc in e0 and e3 leaves almost equal to or higher than HGYHs; but, they have genetic system of weak strength to send enough assimilates to ear for grain filling. Based on the information, the low GYHs were of less efficient strength to utilize leaf chl and N for increasing grain yield which can be easily reflected through the figure 1 and 2A, B & C. Hybrid 8 had N and chl conc either low or medium on the e0 and e3 leaves but not higher than other hybrids. But, it has still been found of having genetic system of strong strength to utilize the leaf chl and N for high grain yield.

Low grain yielding hybrids might have genetic system of weak strength to utilize the N containing proteins, enzymes and chl conc for high grain yield. HGYHs 13 and 6 were having low to medium chl and N conc on the central and top leaves during grain filling. So, they too have genetic system of high strength to utilize chl and N containing protein complexes. HGYHs that had moderate to low N and chl conc imply that they have efficient source-sink correlation. Hybrids 8, 13 and 6 fall in this group from the standpoint of E0SPAD145 Fig 2 A, B & C). Publication of Mae (2004) also reflects that degradation of soluble proteins such as Rubisco releases N to fulfill N demand of developing kernels during crop maturity.

DISCUSSION

In HGYHs, it is a little bit earlier to say that rate of degradation of chl and protein complexes including RUBISCO, efficiency of mobilization of the degraded nutrients to the kernels are relatively functional. The e0 leaf during immediate post-flowering phase might have degraded some protein and chl conc to mobilize towards kernel growth which can be inferred from the sudden decline of the leaf SPAD measure from 95 to 115th day and findings of Dwyer et al., (1995). Since the highest grain yielding performance and high RAT SPAD, chl and N conc in the e0 leaf of the hybrid 8 during earlier grain filling; it can be said that hybrid 8 might have efficient level of fluorescence migration of excitation energy to RC (reaction centers) (shortly abbreviated as FEET) and photosynthetic electron transport (PET) in thylakoid membrane (Fracheboud et al., 1999; Haldimann, 1997; Ribas-Carbo et al., 2000; Verheul et al., 1995) and photosynthetic carbon metabolism (Pietrini et al., 1999); less degradation of photosynthetic pigments (Aroca et al., 2001; Haldimann, 1997, 1999; Leipner et al., 1999); enough and high activities of RUBISCO (Allen and Ort, 2001), Calvin cycle enzymes during flowering and early grain filling in the winter; but the chl and N conc remained almost stagnant throughout the entire period from 95 to 145 DAS.

In HGYHs 8, 12, 11, 13, 5 and 6; small differences in chl and N conc did not cause significant difference in grain yield. So, all the hybrids need not to be explained in detail since variation in chl and N conc is not so large. Hybrids 12, 11 and 6 hesitated to degrade pigment-protein complexes and N-containing proteins and enzymes such as RUBISCO and PEP carboxylase on the e3 leaf, so it might be one of reason of their somewhat lower yields than that of the hybrid 8 (Tables 5A, B; Sup Table 5 A, B, C & D). Alike the hybrid 8; the second HGYH 12 too had similar level of RAT SPAD measure on the e0 leaf. It means that the e3 leaf of the H 12 had optimum efficiency of photochemistry including FEET to RCs and PET during the grain filling.

In HGYHs; efficiencies of N uptake, upward movement of N up to the e3 leaf, maintenance of the nitrogen in different biochemical forms have been found functional to contribute to the grain yield relatively (Table 5A and B and Sup Table 5A, B, C &D). Although it will be earlier, it will be better to say concentration of RUBISCO, Calvin cycle enzymes, conformation of soluble protein complexes and chl might have been functional to contribute to the grain yield in the HGYHs. Furthermore; efficient light harvest, lowest excitation pressure in order to mobilize all the harvested excitation energy into RCs with minimum loss through fluorescence, xanthophyll cycle pool associated NPQ (the phenomena explained by Demmig-Adams and Adams, 1992; Demmig-Adams et al., 1996; Horton et al., 1996) and constitutive NPQ on PSII RC II region (concluded by Huner et al., 1996, 1998; Ivanov et al., 2003, 2006; Sane et al., 2002, 2003; Kramer et al., 2004; Kornyeyev and Hendrickson 2007; Savitch et al., 2009), efficient PET rate, higher quantum yield and efficient CO_2 fixation on e0 to e3 leaves. According to the model explained by Kitajima and Butler (1975), small increase in thermal energy dissipation causes depression in the PET output (Fv/Fm). This phenomenon has also been clarified by Björkman (1987) and Demmig-Adams (1990) from their experiments.

Some other causes of low grain yield can be paired inbreds were less distant parents for superior hybrid combination or for high ‘heterosis’ (Shull, 1948). The leaves of the low yielding hybrids were not so photosynthetic or the manufactured assimilate could not mobilize efficiently to the kernels; or manufactured assimilates were lost through high respiration; or female reproductive organ was not of much strength to attract assimilates; limited number of functional egg cell and fused zygotes and nature of low TKW or asynchronous pollination or asynchronous silk emergence or reduced pollen availability from surrounding population. The hybrids 4 and 3 might have very high excitation pressure in antenna region or high excitation pressure and high constitutive NPQ in PS II RCs II, so the excitation energy could not migrate through antenna pigments to the PS II RCs because of inefficient PQ pool or high proportion of non-functional RCs (Savitch et al., 2009), or less efficient PET components.

A variety of pigment complexes in the antenna region participates in light harvesting, excitation energy transfer to the RC through FEET and photosynthesis regulation. Furthermore, different forms of carotenoids such as violaxanthine, anteraxanthine, zeaxanthin and lutein known as xanthophyll cycle pool (Toth et al., 2002; Haldiman, 1999) participate in excess energy dissipation if chl level could not send excitation energy to final RCs or under stresses of suboptimal temperature (Haldiman 1999; Savitch et al., 2009). Xanthophyll cycle pool protects the chl-protein complexes and other photosynthetic apparatuses from photooxidative damage through dissipation of the excess of excitation energy in thermal form ‘NPQ’ under stressful conditions (Schmid, 2008). In addition, the loss of excitation energy might be because of lower proportions of functional RCs in photosystem cores (Savitch et al., 2009).

During terminal grain filling of different hybrids; chl and N conc implying SPAD measure has been found below threshold (40, Netto et al., 2002; 2005) (Table 4.3.4.B). The SPAD below 40 indicates impairment of efficiency of photochemistry of PS II based PET rate (Netto et al., 2002; 2005) and FEET (Förster, 1948) from antenna pigments to RC chl a molecules. In the hybrid 8; low level of chl and N conc in e3 leaf among the top five HGYHs, terminal degradation of chl and protein complexes has been found functional to contribute to grain filling (Table 5A, B; Sup Table 5A, B, C & D). The HGYHs might have highly efficient photosynthetic apparatus on e0 than the e3 leaf; and efficient leaf nutrient mobilization efficiency from protein degradation to the kernels during crop maturity (Feller et al., 2008). Besides, it can also be said that non-collinear correlation between the SPAD and grain yield can also reflect differential strength in N pulling, chl synthesis, chl and soluble protein degradation among different leaves and different genetic system of the hybrids. Some hybrids send N to kernels through leaves, some send through root uptake directly, some utilize both systems to send N to the kernels. And there is still different strength of different leaves even in the same hybrids on the matters to degrade complex N containing biochemical and send N to the kernels if they are mobilizing N through the leaves. Differential SPAD, chl and N conc in two leaves and different stages of grain filling in reference to differential grain yielding potentiality of the different hybrids reflect such interpretations through mean comparison DMRT tables.

Highest rate of leaf pigment and protein complex degradation on the e0 leaf from 145 to 155 or terminal grain filling and their effective and active mobilization towards sink might have favored for the highest grain yield on HGYHs. In HGYHs, e3 leaf has been found with less SPAD; so, the HGYHs had low efficiency to harvest red photon and most of the PS II and downstream electron acceptors were in the oxidized state in e3 leaf. Low chl, low N but high carotenoid conc can exist in the e3 or taller leaves. Low SPAD and low N conc also imply low protein complexes to coordinate between chl and car pigments to transmit heat energy from chl triplets to car triplets (Siefermann-Harms, 1987) and to dissipate much energy in thermal form through molecular vibrations. This can be one of the possibilities that can happen on the e3 leaf. Besides; e3 or taller leaves of the hybrids can be said of lower photosynthetic strength than the e0. But; the hybrids had to dissipate more harvested light energy in thermal form to protect photosynthetic apparatus from oxidative damage on the e3 and taller leaves than the e0. So, inefficient heat dissipation also damages photosynthetic apparatuses larger in e3 or taller leaves than e0 leaf and lower few leaves below e0. Above explanation of hybrids from the standpoint of leaf RAT SPAD and grain yield imply that variation exists among maize hybrids for efficiency of plant body N utilization for functional grain yield components and grain yield. Experiments with the US Corn-Belt (Balko and Russel, 1980), tropical (Muruli and Paulsen, 1981; Lafitte and Edmeades, 1994; Banziger et al., 1997), and European maize (Bertin and Gallais, 2000) also indicated that genotypes can differ considerably in their N-use efficiency. Hence, breeding for adaptation and evaluation of maize genotypes are possible for efficient N utilization under low N level.

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Supplemental Information

Pedigree of research materials, climate, chlorophyll and nitrogen concentrations

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Table 1: Outline of the experimental trial of the winter hybrid maize

Design: Randomized complete block design (RCBD)
Replications: 3
Net plot size: 3 meter x 1.4 meter (L x B) = 4.2 m^2
Spacing: 0.70 x 0.25 m^2
Planting date: October 03, 2012
Harvest date: April 06, 2013 (Crop duration 185 days)

Table 2: Treatment details of the winter hybrid maize trial A$_1$

Entry	Hybrids	Entry	Hybrids	Entry	Hybrids
1	RML-19/NML-2	6	RL-111/RL-189	11	RML-57/RML-6
2	RL-137/RL-168	7	RML-95/RML-9	12	RL-170/RL-111
3	RML-55/RL-29	8	RML-86/RML-96	13	RL-154/RL-111
4	RL-99/RL-161	9	RL-36/RL-197	14	RML-4/NML-2
5	RML-6/RML-19	10	RL-180/RML-5	15	Gaurav (For check)

Table 3: Pooled variance analysis of the leaf RAT SPAD, chl and N. The table reflects dynamics of e0 and e3 leaf SPAD, chlorophyll and nitrogen during grain filling

	|-------------SPAD-------------|			|----------CHLO[C]---------|		|--------Nitrogen [D]-------|	
Source[1]	DF	MS	PROBA	MS	PROBA	MS	PROBA
Replication	2	62.886		156.447		0.622	
Hybrids (A)	14	278.68	0.0252	600.938	0.0372	2.371	0.0311
Error	28	117.549		273.62		1.042	
Leaves (B)	1	6148.907	0	14498.309	0	49.302	0
AB	14	156.733	0	310.005	0.0001	1.369	0
Error	30	27.941		58.207		0.236	
Ages (C)	6	983.793	0	1986.953	0	56.751	0
AC	84	9.143	0.184	18.08	0.1238	0.123	0.0027
BC	6	18.426	0.0319	67.813	0.0002	0.689	0
ABC	84	9.443	0.1369	16.587	0.2615	0.109	0.0211
Error	360	7.9		14.972		0.078	

[1] Factor A for hybrids; Factor B for leaves: e0 and e3; [C] chlorophyll content µg cm^{-2} computed using equations of Cerovic et al. 2012; and [D] N estimated using equation of Dwyer et al. (1995) mentioned in Table 3.3.1.

Table 4: Mean square values and significance of leaf SPAD, chl and N. Leaf chl and N dynamics on e0 and e3 leaves are shown in different stage of crop plants at ten days interval during grain filling

		|--------------E0 LEAF	-------------	-|	|--------------E3 LEAF	-------------	-|
SOV	DF	SPAD	CHL[C]	Nitrogen[D]	SPAD	CHL[C]	Nitrogen[D]
Replication	2	90.661	213.725	0.734	28.757	78.938	0.321
Hybrid (A)	14	92.831	242.403	0.97	342.582**	668.54**	2.77**
Error	28	70.409	179.238	0.69	73.039	147.017	0.574
Times (C)	6	604.965**	1342.238**	34.605**	397.254**	712.527**	22.835**
AC	84	8.381	17.366	0.097	10.205**	17.301**	0.134**
Error	180	10.59	20.33	0.107	5.209	9.614	0.049

[Z] Cerovic et al. (2012); [D]Dwyer et al. (1995).

*Significant at 0.05 and ** very significant at 0.01 level of probability.

Table 5A: DMRT of hybrids for leaf RAT SPAD, chl and N

Entry	Grain yield t ha^{-1}	E0SP95	E0SP105	E0SP115	E0SP125	E0SP135	E0SP145	E0SP155
8	12.54 A	55.4	53.4	53.2	55.5	55.3 A	54.3 AB	42.6
12	11.80 A	55.0	53.3	52.6	52.6	53.0 ABC	53.6 AB	45.3
11	11.55 A	55.5	54.2	52.2	53.7	53.5 AB	57.3 A	51.5
13	11.31 AB	53.0	51.0	48.4	49.3	46.6 C	46.7 C	35.2
5	11.05 AB	55.0	52.2	50.5	52.3	50.6 ABC	51.3 BC	41.4
6	11.02 AB	52.7	53.0	50.2	51.0	49.2 ABC	48.6 BC	34.8
10	9.78 ABC	55.9	55.3	52.1	52.4	52.2 ABC	53.7 AB	48.6
1	9.75 ABC	56.6	54.4	53.6	51.9	51.5 ABC	52.5 ABC	42.8
7	9.70 ABC	51.3	50.2	47.3	49.6	50.7 ABC	50.9 BC	40.2
14	9.64 ABC	53.4	50.6	51.1	50.4	50.0 ABC	50.9 BC	45.8
2	9.47 ABC	54.4	51.7	49.6	49.6	49.7 ABC	49.0 BC	39.6
9	9.30 ABC	55.3	55.5	51.6	52.5	50.1 ABC	51.8 ABC	46.1
15	**9.17 ABC**	**52.4**	**50.7**	**49.2**	**48.9**	**48.4 BC**	**49.3 BC**	**41.8**
4	7.87 BC	56.7	53.2	53.0	54.2	52.8 ABC	51.1 BC	45.5
3	7.03 C	53.8	50.8	47.8	49.4	48.7 ABC	46.9 C	41.0
Mean	10.07	54.4	52.6	50.8	51.5	50.8	51.2	42.8

Maize hybrids and their entries are RML-19/NML-2 (1), RL-137/RL-168 (2), RML-55/RL-29 (3), RL-99/RL-161 (4), RML-6/RML-19 (5), RL-111/RL-189 (6), RML-95/RML-96 (7), RML-86/RML-96 (8), RL-36/RL-197 (9), RL-180/RML-5 (10), RML-57/RML-6 (11), RL-170/RL-111 (12), RL-154/RL-111 (13), RML-4/NML-2 (14) and Gaurav (15). Hybrids are arrayed from top to bottom based on decreasing grain yielding potentiality.

Table 5B: DMRT of hybrids for leaf RAT SPAD, chl and N. Fifteen hybrids were in the trial A1.

Entry	E3SP95		E3SP105		E3SP115		E3SP125		E3SP135		E3SP145		E3SP155	
8	48.3	A	44.2	A-D	42.7	B-E	43.7	BC	43.8	CDE	43.4	BC	32.5	FGH
12	48.3	A	49.9	A	49.1	AB	51.9	A	52.6	A	49.5	AB	45.5	ABC
11	50.3	A	49.6	AB	49.9	A	50.5	AB	52.1	AB	52.2	A	47.8	A
13	47.5	A	46.4	ABC	45.3	A-D	46.6	ABC	46.1	A-E	46.8	ABC	41.7	A-E
5	50.1	A	47.3	ABC	45.2	AD	46.5	ABC	45.2	B-E	46.0	ABC	42.9	A-D
6	47.2	A	46.7	ABC	45.4	A-D	47.4	ABC	46.2	A-E	44.9	ABC	38.6	B-F
10	50.2	A	48.3	AB	45.9	A-D	46.2	ABC	45.9	A-E	45.3	ABC	38.4	B-F
1	47.4	A	43.5	A-D	42.6	B-E	41.4	CD	41.5	DEF	40.6	C	36.1	D-G
7	40.0	B	38.5	D	37.5	E	37.1	D	35.5	F	32.3	D	25.4	H
14	44.3	AB	42.7	BCD	40.4	CDE	41.2	CD	39.7	EF	39.1	CD	28.9	GH
2	48.6	A	48.9	AB	49.7	AB	49.5	AB	50.2	ABC	49.0	AB	47.0	AB
9	49.3	A	48.6	AB	47.1	ABC	45.6	ABC	47.4	A-D	44.5	ABC	34.7	D-G
15	**41.2**	**B**	**40.7**	**CD**	**39.7**	**DE**	**42.2**	**CD**	**43.6**	**CDE**	**40.9**	**C**	**32.9**	**E-H**
4	48.4	A	47.1	ABC	45.8	A-D	46.2	ABC	44.1	CDE	41.6	BC	40.8	A-F
3	47.0	A	45.6	ABC	46.1	A-D	46.1	ABC	45.3	B-E	43.5	BC	37.1	C-G
Mean	47.2		45.8		44.8		45.5		45.3		43.9		38.1	

Table 6A: Correlation coefficient between grain yields and RAT related traits. The coefficient are between grain yield and e0 and e3 leaf SPAD value, chlorophyll and nitrogen content at ten days interval during grain filling (45 plots average values were used to estimate the r).

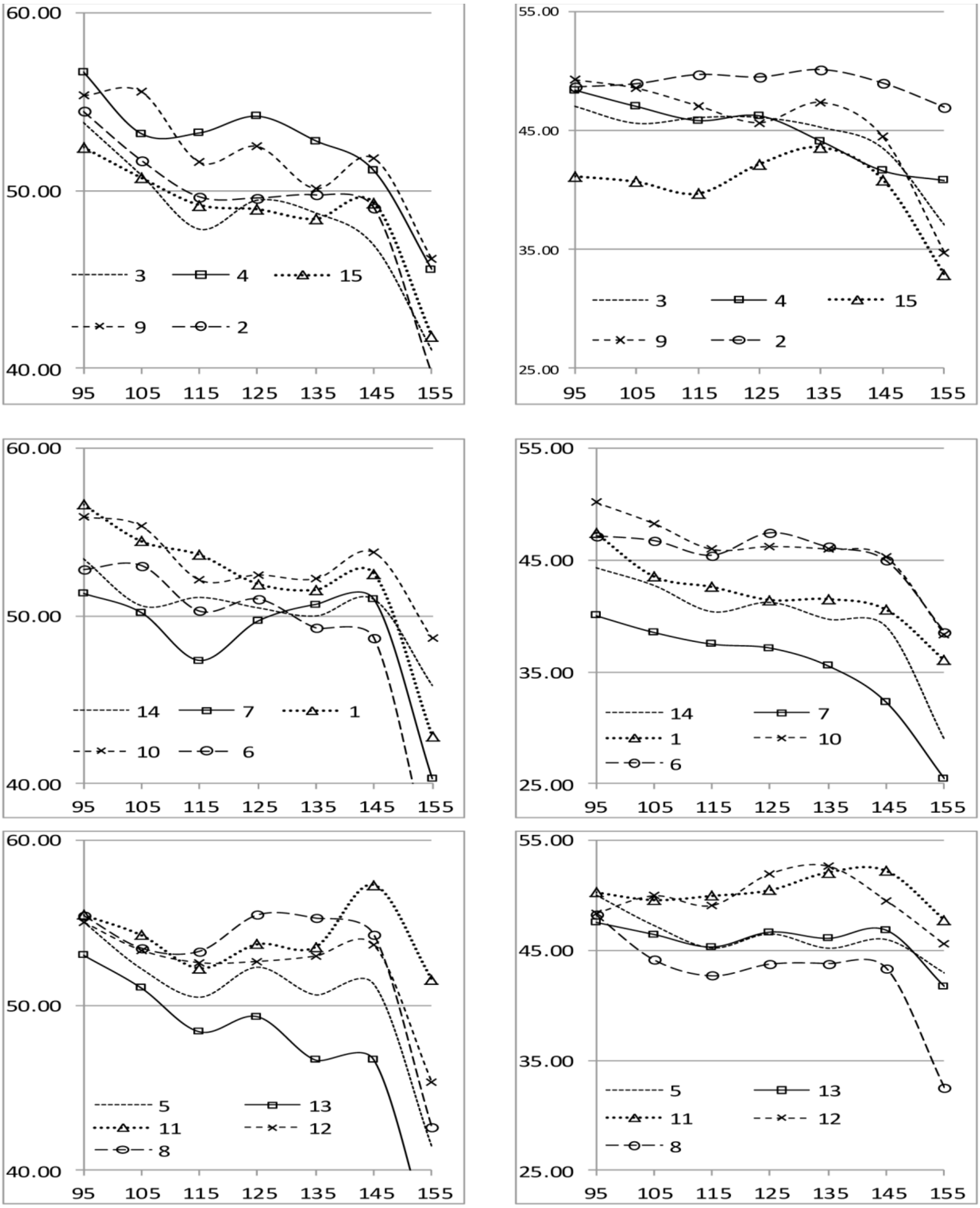
	E0	E0	E0	E0	E0	E0	E0
Time	95	105	115	125	135	145	155
SPAD	0.359*	0.407**	0.371*	0.431**	0.512**	0.504**	0.352*
Chlo	0.347*	0.402**	0.361*	0.433**	0.513**	0.493**	0.385**
N	0.353*	0.395**	0.373*	0.432**	0.511**	0.504**	0.353*

*It is significant at 0.05 level; **very significant at 0.01 level

Table 6B: Correlation coefficient between grain yields and RAT related traits. The coefficient are between grain yield and e0 and e3 leaf SPAD value, chlorophyll and nitrogen content at ten days interval during grain filling (45 plots average values were used to estimate the r).

	E3	E3	E3	E3	E3	E3	E3
Time	95	105	115	125	135	145	155
SPAD	0.191	0.31*	0.266	0.365*	0.432**	0.406**	0.285
Chlo	0.189	0.301*	0.259	0.371*	0.441**	0.41**	0.308*
N	0.202	0.304*	0.268	0.369*	0.433**	0.404**	0.285

*It is significant at 0.05 level; **very significant at 0.01 level



DAS	95	105	115	125	135	145	155
GDD	725	718	743	785	839	910	1013
n/x	2/22	0/19	4/21	4/25	6/25	9/26	10/31

DAS	95	105	115	125	135	145	155
GDD	725	718	743	785	839	910	1013
n/x	2/22	0/19	4/21	4/25	6/25	9/26	10/31

Fig 1: Graphs to demonstrate dynamics of RAT SPAD measures of the fifteen hybrids. RAT SPAD measures in Y axis with respect to days after sowing (DAS 95, …, 155), growing degree days (GDD) and average minimum/maximum temperature (n/x) in each last ten days. on X axis. Left column is for e0 and right column is for e3 leaves. Hybrids 3, 4, 15, 9, 2, 14, 7, 1, 10, 6, 5, 13, 11, 12 and 8 are in order of increasing grain yield.

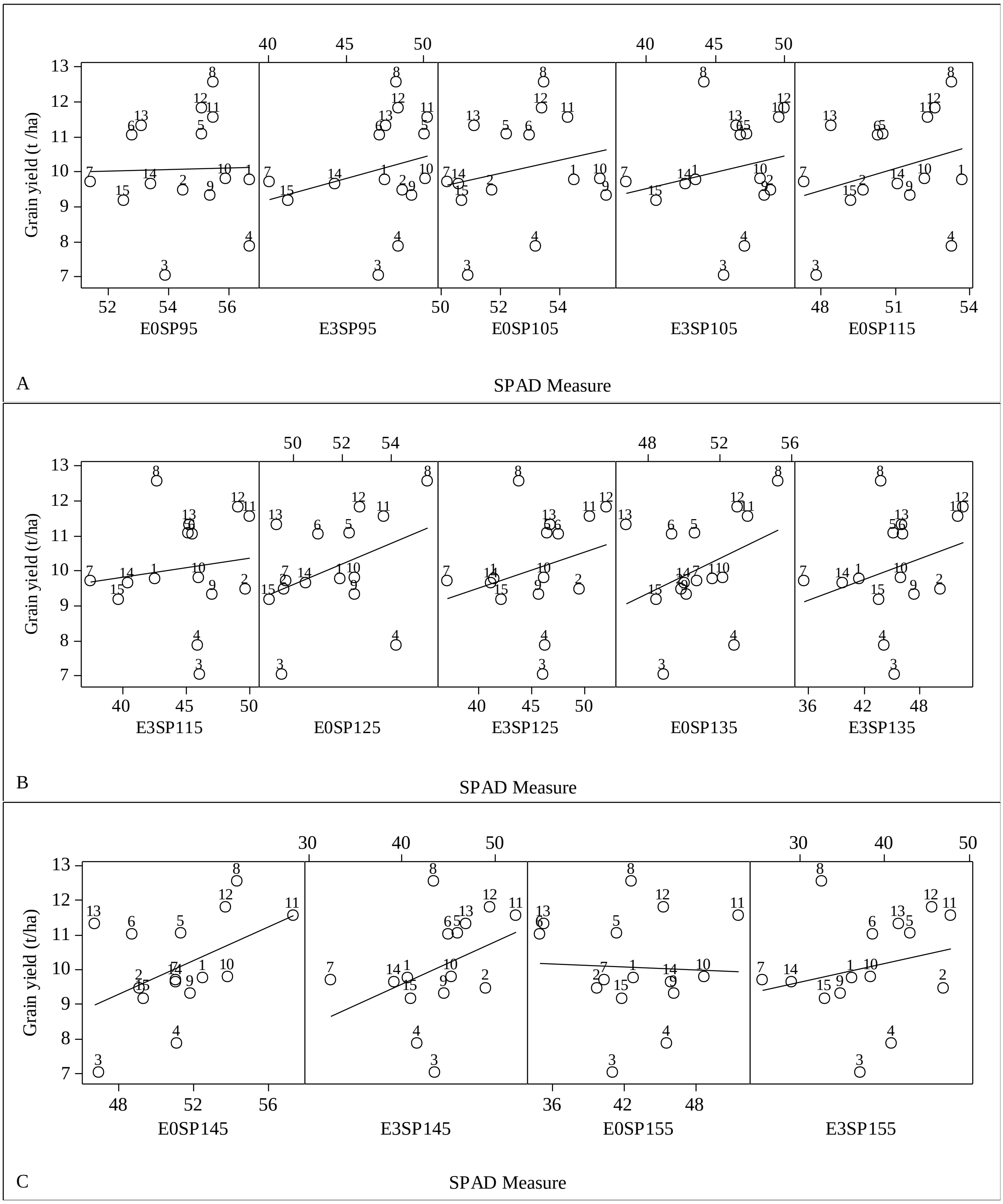


Figure 2A-C: Linear regression line between grain yield and SPAD. The SPAD measures are on with E0 and E3 leaves of the maize hybrids.(A) For 95 to 115 DAS (B) For 115 to 135 DAS (C) For 145 to 155. Maize hybrids and their entries are RML-19/NML-2 (1), RL-137/RL-168 (2), RML-55/RL-29 (3), RL-99/RL-161 (4), RML-6/RML-19 (5), RL-111/RL-189 (6), RML-95/RML-96 (7), RML-86/RML-96 (8), RL-36/RL-197 (9), RL-180/RML-5 (10), RML-57/RML-6 (11), RL-170/RL-111 (12), RL-154/RL-111 (13), RML-4/NML-2 (14) and Gaurav (15).