Stay green traits of hybrids of maize (*Zea mays* L.) below and above ear during grain filling and their association with grain yields in winter in subtropical foot plain of Nepalese Himalaya

Stay green traits in maize

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 (IAAS), Post-Graduate Campus, Tribhuvan University, TU Kirtipur Boundary, Kathmandu, 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]Nepal Agriculture Research Council (NARC), Singhdarawar Plaza, Kathmandu, Nepal.

[*]Corresponding author: navraj.adhikari@gmail.com

ABSTRACT

Introduction: Plant breeders are in search of traits for high grain yielding hybrids (GYHs), genotypes or populations in several crop species including maize. So, the research has been designed to examine a variety of SG traits during entire grain filling (GF) and their association to grain yields (GYs) among the fifteen newly bred single cross maize hybrids. **Materials and methods**: An RCBD trial of three replications was conducted planting seeds of the hybrids on October 3, 2012 at Rampur, Chitwan in the subtropical foot plain of Nepalese Himalaya. The sowing date of the trial was arranged to expose flowering and grain filling to natural winter and to represent the commercial hybrid maize production season in granary belt. **Results and discussion:** Although previous studies reported that leaves that stay green long have been found positively grain yield attributing; this study have depicted either long, or short stay-green (SG) are high grain yielding. Polynomial regression equations (PREs) obtained between GYs and numbers of green leaves below ear (BtmGrn) on 110, 125 and 140th day during GF of the FHs (fifteen hybrids) had coefficient of determination (r^2) less than 18%. Although it is so, there is still two diagnostic association curves for high GYHs 8, 12, 11, 13, 5, 6 and 7 and medium to low GYHs 10, 1, 14, 2, 9, 15, 4 and 3 separately. After the date, high GYHs still displayed association curve between GYs and BtmGrn. In addition; PREs obtained between GYs and numbers of green leaves above ear (TopE0Grn) during entire GF of the FHs had r^2 19 to 53%. Although it is so, there is still separate diagnostic association curve for high GYHs 8, 12, 11, 13, 5, 6 and 7; and medium to low GYHs 10, 1, 14, 2, 9, 15, 4 and 3. High GYHs and medium to low GYHs similarly displayed different association curve between GYs and numbers of total green leaves (GrnLves) on 110 to 155th day during entire GF and that had r^2 equal to 21 to 53%. Although r^2 of the PRE formed between GYs and days for net ear senescence duration (ErSenDur) has been found higher than PREs formed for days for ear senescence completion (ErSenCmp) and net ear

senescence duration (ErSenDur); two meaningful separate quadratic curves can form between ErSenIni and GYs of high GYHs and medium to low GYHs. Curve formed between GYs and days for 0% green PP (plant population) (days for 100% PP senescence) of the FHs reflect strong positive correlation and almost straight line regression among fourteen hybrids except the highest GYH 8. Although the hybrid 8 lost SG almost earliest; but, its GY has been found highest. It means that the hybrid had highly photosynthetic leaves to contribute to high GY. But; fourteen high to low GYHs are inside the strong positive correlation pattern except GYH 8. So, number of days required by hybrids for 0% SG PP can be positive marker for selection of high GYHs.

Abbreviations used

SG= stay-green; PP=plant population; GF = grain filling duration; GY= grain yield; GYHs= grain yielding hybrids; RCBD=randomized complete block design; PRE(s) = polynomial regression equation(s); DMRT= Duncan's multiple range test; NARC = Nepal Agriculture Research Council; H(s) = hybrid(s); NADPH = Nicotinamide adenine diphosphate in reduced state [energy molecules from PET (photosynthetic electron transport) chain]; ATP = Adenosine triphosphate; ErSenIni= average numbers of days for ear senescence initiation for randomly selected plants; ErSenCmp=average numbers of days for ear senescence completion; ErSenDur= net ear senescence days.

INTRODUCTION

Previous reports stated that GYs of some crops are highly, positively and proportionally correlated to rate of photosynthesis. But photosynthesis declines if the maize plants lose SG leaf prematurely or leaves undergo senescence prematurely (Tollenaar and Daynard, 1978; Wolfe et al., 1988a, b). Senescence is losing greenness from leaves and plant parts and it can occur in stage of plant life; whereas senescence during plant aging is a special phenomenon (Leshem et al., 1986). Sexton and Woolhouse (1984) stated that ageing of annual crops includes physiological changes in reference to time and phase of plants which must occur after flowering for fruit or kernel set. Terminal phase of the crop maturity is plant death that occurs from the result of the exhaust of vital nutrients from the plant parts for mobilization to the flowering and seed or fruit setting. Here, the type of senescence that occurs after flowering and seed set is accelerated plant physiological phenomena and it is the scope of the paper.

Spano et al. (2003) displayed that elongation of GF boosts grain yield of crops. Elongation of GF is only possible if the duration of SG is elongated delaying leaf senescence. Ma and Dwyer (1998) displayed evidence that maize hybrid with the trait of longer SG and longer GF after flowering produced 24% more dry matter and assimilated 20% more nitrogen than early SG losing hybrid during GF. Thomas and Howarth (2000) showed that delaying start of losing SG 'leaf senescence' for two days increased fixed CO_2 by 11% in *Lolium temuletum*. Similar science of increased CO_2 fixation came into existence from the crop research of tobacco (*Nicotiana tabacum*) and sorghum (*Sorghum bicolor* L) (Gan and Amasino, 1995; Borrell et al., 2000).

SG trait and senescence phenomena have been studied in maize in depth at biochemical to molecular and advanced physiological level. And still such studies are going on. But, SG traits have not been studied and reported in search of marker traits for maize breeding. So, the paper also includes these aspects. The section includes how high versus low grain yielding newly bred single cross hybrids of yellow maize behave to contribute to GYs of the FHs through a variety of SG traits such as numbers of bottom and top green leaves during GF, days for senescence of bottom leaves, top leaves, percent greenness of PP on 120th day during peak GF, days for senescence of PPs during terminal GF and crop maturity to contribute to

GYs of the FHs. The work has also discovered GY estimating equations from the standpoint of the above mentioned SG traits and three traits of ear senescence of the FHs for discovery of GY estimating PREs. It also includes discussion of genetics of SG traits in the FHs from the standpoint of phenomena of SG, senescence and GYs.

MATERIALS AND METHODS

Experimental site and treatment details

Newly bred fifteen single cross hybrids of normal yellow maize were examined conducting an RCBD trial of three replications for their SG traits from the same trial as mentioned by Adhikari et al. (2015a) and briefly listed in Table 1 in the paper. Pedigrees of the FH have been shown by Adhikari et al. (2015 b). The trial was conducted in the research field of National Maize Research Program (NMRP) in Nepal Agriculture Research Council (NARC) which is located in the longitude $27^0$37'N, latitude $84^0$24'E and altitude 228 m above sea level. That is, this research site is in subtropical foot plain of Nepalese Himalaya. Sandy loam top soils of the research field have pH range 5-5.5. For a standard check, Gaurav (hybrid entry 15), a newly released single cross hybrid cultivar was put into the trial. Climate details in the subtropical research site in the trial period have been summarized by Adhikari et al. (2015b).

Crop management in the trial

Organic manure was first applied @ 33 t ha^{-1} before the start of land plowing. The field was finely ploughed and made clod free. Application of chemical fertilizers was done @ 120:60:40 kg N, P_2O_5 and K_2O ha^{-1}. All amounts of the phosphorous and potassium fertilizers were in the form of DAP and murate of potash as basal dose. N was applied in the form of urea in split doses of 50: 25: 25 % as basal, 45 and 60 DAS respectively. Seeds of the hybrids were planted manually on October 03, 2012. Two seeds were dropped in each hill in a net spacing of 0.25 m for hill to hill distance x 0.70 m for row to row distance in each of the two rows of each net plot size of 3 x 1.4 m^2. Blocks were separated with an alley of 1 m. The row direction was on north-south. Twenty four plants were maintained in each two-row plot in order to maintain plant population density of 57,143 plants ha^{-1} on 30^{th} day which was the time of weed removal and soil loosening. Soils were raised on 45 DAS to cover prop roots well and make plants firm. Four irrigations were done through depression between rows on 50, 70, 90 and 110 DAS through shallow tube well of 4" pipe. The crop material was harvested on 185^{th} day.

Traits and their observations

Traits considered to study dynamics of green leaves are average of total numbers of green leaves below and above ear (BtmGrn110, BtmGrn125, BtmGrn140, BtmGrn155, BtmGrn170; TopEoGrn110,TopEoGrn125, TpE0Grn140, Tp+E0Grn155, and Tp+E0Grn170 respectively) of randomly selected five plants in each plot below ear when crop was 110, 125, 140, 155 and 170 days old. Average total numbers of green leaves below and above ear leaf including ear leaf per plant were computed as the sum of the leaves below and above ear from each of fifteen randomly selected plants in the fifteen days interval during grain filling (GrnLvs110, GrnLvs125, GrnLvs140, GrnLvs155, and GrnLvs170).

Furthermore; days for SG leaves of 50% and 100% population (50%LFGRN, 0%LFGRN) and days for SG plant of 50 and 100% population (50%POPGRN and 0%POPGRN), days for ear senescence initiation, ear senescence completion were recorded from five randomly selected plants in each plot and average of the five plants computed for plot value of the trait. Days for ear senescence initiation (ErSenIni), days for ear senescence completion (ErSenCmp), average net ear senescence duration in days (denoted by

ERSENDURA in uppercase) were also computed from the randomly selected five plants per plot. It was done through five randomly selected plants in each plot. Data analysis and graphs plotting have been done and shown.

RESULTS

Variance analysis of the trial

The FHs that matured in almost 180 days in winter in subtropical foot plain of Nepalese Himalaya have been found highly significant different from the standpoint of total green leaf numbers during early to mid GF; but only significant different in terminal crop maturity. In other words, significant differential total green leaf numbers prevailed among the FHs during GF. If variance analysis of green leaf numbers below and above ear has been examined separately; hybrids can be differentiated significantly from the standpoint of green leaf numbers below ear during early GF; but, non-significantly different during late GF. Furthermore, the hybrids have been found significant different from the standpoint of bottom green leaf numbers on 110, 125 and 140. About the remarkable leaf senescence rate; hybrid wise significant differential leaf senescence rate of bottom green leaves was in the duration from 140 to 155th day, it is terminal GF. From the standpoint of top green leaf numbers; hybrids have been found significant different on 110, 125, 140, 155 and 170. It means that there was hybrid wise significant differential highest senescence rate of top green leaves in the duration from 155 to 170th day (Appendix 4.8.1A-C). In addition; from the DMRT, hybrids have been found significant different from the standpoint of the traits of days for 50% leaf green in plots, 0% SG leaves in plots, 50% population SG, 0% population SG (based on plot data); days for ear senescence initiation, ear senescence completion and ear senescence duration (Supplementary file, Sup Table 1, 2 and 3).

Evaluation of the maize hybrids from standpoint of grain yield and SG traits

High GYH 8 (RML -86/RML-96) is characterized through the green leaves and SG traits during grain filling senescence as having optimum value of the traits of bottom green leaves on 110, 125, 140 and 155th day among the FHs. Similarly, H8 had intermediate numbers of top green leaves when the crop was 110, 125, 140 and 155 days old. But; after 155 days, top green leaves declined sharply. It means that the H 8 had quicker leaf senescence at terminal maturity among the FHs. The H 8 had less number of total green leaves from 110 to 140; and then it maintained least numbers of green leaves on 155 than the other five high GYHs. This way high GYH 8 declined green leaves sharply. Since the hybrid is the highest grain yielding; it can be concluded that the small numbers of green leaves in overall evaluation of the H 8 were of higher photosynthetic efficiency than other hybrids (Table 5, 6 & 7). Hybrid 8 took short duration for ear initiation, completion and net duration for ear senescence, shortest duration for 50% leaf senescence, 100% leaf senescence of the plots, days for 50%, 100% PP senescence, and shortest duration for all leaves senescence of the plot (Table 3, 4, 5, 6 and 7).

Second highest GYH 12 had longer SG leaves and slow leaf senescence than H 8. It can also be concluded that the H 12 maintained photosynthetic apparatus and greenness for longer duration in rising temperature during transition from winter to spring and grain filling to terminal crop maturity. It might be because H 12 had efficient xanthophyll cycle in the top leaves to protect green pigment-protein complex longer. The H 12 was earlier to initiate the ear senescence, ear senescence completion, but took 2 days longer for ear senescence duration of the entire ear. The hybrid took longer duration for senescence of all the leaves than the average of the FHs. Besides, the 50 and 100% population of the H 12 stayed green longest among the FHs (Figure 1, 2, 4 & 5; Table 3, 4, 5, 6 & 7).

Third highest grain yielding H 11 kept larger numbers of above ear green leaves for long duration. Since the hybrid had large numbers of green leaves, it had tendency to keep leaf greenness for longer duration. It also imply that the H 11 kept photosynthetic apparatus such as PS II, PS I, NADP reductase, ATP synthase, soluble proteins, Calvin cycle enzymes including RUBISCO and PEPC longer, high efficiency to maintain plant water checking the transpiration loss, high PSII PET potentiality, high photochemical quenching and optimal NPQ to protect the green pigment and photosynthetic apparatus. The H 11 was a little bit late to initiate and complete senescence of the ear; but, it took longer net duration for ear senescence. The H 11 stayed green leaves long from the standpoint that it took longest duration to turn all green leaves into ash (of terminal 50 to 100% population) (Table 3, 4, 5, 6, 7; Fig 1, 2, 3, 4 & 5).

Among the three high GYHs: 13, 5 and 6; H 5 has been characterized with larger numbers of bottom green leaves as well as top green leaves from 110 to 155 DAS during GF than Hs 13 and 6; but the Hs13 and 6 lost bottom and top green leaves rapidly after 155 DAS. It can be discussed to conclude that H 5 had more efficient mechanism to maintain green leaves below and above the ear leaf than the Hs 13 and 6 during GF. It can be concluded that the hybrids 13, 5 and 6 have been found high grain yielding. Although it will be a little bit earlier to explain the reason of high grain yielding, it will be better to include the possible scientific explanation that the leaves of the hybrids 13, 5 and 6 had efficient potentiality to trap light energy through green pigment-protein complexes, efficient mechanism in PS II to dissociate water into protons, electrons and molecular oxygen and transfer the protons and electrons for formation of energy molecules: NADPH, ATPs and reduced ferrodoxin to turn inorganic carbon into triose sugar and its derivatives; and high efficiency of phloem loading-unloading to final sink through the consumption of the NADPs and ATPs (Table 3, 4, 5, 6, 7).

The leaves of the hybrids 8, 13 and 6 were very functional to contribute to grain filling (Fig 1, 2 & 3). The hybrids 13 and 6 had less number of top green leaves from 110 to 125 DAS and then maintained the green leaves till 155 DAS. But, H 13 maintained highest numbers of top green leaves on 170 DAS (Table 5, 6 & 7; Fig 1). Among the intermediate grain yielding hybrids 10, 1, 7, 14, 2, 9 and 15; H 1 were having highest numbers of bottom green leaves and high numbers of top green leaves, but very high numbers of total green leaves during most of the GF. Among the intermediate grain yielders; hybrid 2 had lowest numbers of bottom green leaves but intermediate numbers of top green leaves (Table 3, 4, 5, 6 and 7).

Dynamics of SG leaves below and above the topmost ear during GF

Numbers of green leaves that stayed green below the topmost ear was in straight line pattern in most of the hybrids in the winter in the subtropical region. But, the dynamics of green leaves number above the topmost ear has been have been found a little deviated from the straight line equations (Table 2).

SG trait comparison for grain yield marker

A variety of SG traits have been used to discover PREs and curves to examine them whether some traits can be breeder's marker for selection of high GYHs during breeding and selection. Numbers of green leaves below ear (BtmGrn) in the hybrids on five dates during GF could not give reliable regression equations for grain yield determining marker since all PREs were with r^2 less than 20% (Equations from 1 to 5). Numbers of green leaves above ear (TopE0Grn) in the hybrids on five dates during GF could give more reliable PREs for grain yield determining marker since all the equations were with r^2 from 47 to 53% (Equations from 6 to 10). Numbers of total green leaves below and above ear (TopE0Grn) in the hybrids on five dates during GF could also give more reliable PREs for grain yield determining marker since all the equations were with r^2 up to 53% (Equations from 11 to 15).

The regression equations discovered are the following: Grain yield in t ha^{-1} is designated by Y

$Y = -131.1 + 73.3X - 12.57X^2 + 0.712X^3$; r-sq = 9.0% (where X = BtmGrn110) (1)
$Y = -18.1 + 11.96X - 1.55X^2 + 0.056X^3$; r-sq = 11.6% (where X = BtmGrn125) (2)
$Y = -13.3 + 10.7X - 1.31X^2 + 0.019X^3$; r-sq = 17.0% (where X = BtmGrn140) (3)
$Y = -6.78 + 24.56X - 11.40X^2 + 1.673X^3$; r-sq = 12.7% (where X = BtmGrn155) (4)
$Y = 10.46 - 22.64X + 111.8X^2 - 140.7X^3$; r-sq = 13.4% (where X = BtmGrn170) (5)

$Y = -1470 + 632.5X - 89.85X^2 + 4.244X^3$; r-sq = 53.3% (where X = TopEoGrn110) (6)
$Y = -819 + 359.7X - 52.01X^2 + 2.506X^3$; r-sq = 47.6% (where X = TopEoGrn125) (7)
$Y = -682.7 + 304.9X - 44.77X^2 + 2.194X^3$; r-sq = 48.5% (where X = TopEoGrn140) (8)
$Y = 42.32 - 24.38X + 5.626X^2 - 0.4079X^3$; r-sq = 32.1% (where X = TopEoGrn155) (9)
$Y = 13.08 - 8.700X + 5.665X^2 - 1.016X^3$; r-sq = 18.8% (where X = TopEoGrn170) (10)

$Y = -218.8 + 49.2X - 3.51X^2 + 0.0826X^3$; r-sq = 23.2% (where X imply GrnLvs110) (11)
$Y = -108.7 + 21.9X - 1.21X^2 + 0.0178X^3$; r-sq = 37.2% (where X imply GrnLvs125) (12)
$Y = -841.9 + 228.9X - 20.45X^2 + 0.6072X^3$; r-sq = 52.6% (where X imply GrnLvs140) (13)
$Y = -19.25 + 10.51X - 1.217X^2 + 0.0454X^3$; r-sq = 28.7% (where X imply GrnLvs155) (14)
$Y = 13.49 - 8.813X + 5.182X^2 - 0.8457X^3$; r-sq = 20.9% (where X imply GrnLvs170) (15)

During crop maturity phase; days for 50 % population green gave equation with highest r^2 (=54%). Then, grain yield estimating equations extracted from days for 0% leaf green in hybrid PPs and percentage of plot green above ear have also high r^2 (=39.4). Equations obtained from days for 50% leaf green, days for 0% green PP and percent bottom leaf senescence in the plot on 120th day were with less r^2. Polynomial equations obtained from days required for initiation, completion and net duration of ear senescence taken by five randomly selected plants in each of the plots had comparatively low r^2. Among the three traits; equations obtained from net duration for ear senescence have somewhat higher r^2 than the days for initiation and completion of ear senescence. It also implies that duration of ear senescence can also imply abnormal or optimal physiology of grain yield contributing phenomena. In contrast to it, polynomial curve constructed between grain yields and days for ear senescence initiation also shed light to classify hybrids into two types: highly responsive and less response of grain yields.

The regression equations discovered are the following: Grain yield in t ha^{-1} is designated by Y

$Y = 410801 – 7934X + 51.08X^2 - 0.1096X^3$; r-sq = 21.2% (Where X=50%LfGrn). (16)
$Y = -526149 + 8937X - 50.60X^2 + 0.0955X^3$; r-sq = 39.6% (Where X=0% LfGrn). (17)
$Y = 282749 – 4960X + 29.00X^2 - 0.05652X^3$; r-sq = 54.3% (Where X= 50%PopGrn(18)
$Y = -61688 + 1034X - 5.78X^2 + 0.01077X^3$; r-sq = 30.7% (Where X=0% PopGrn). (19)
$Y = -1045 + 34.74X - 0.3817X^2 + 0.00139X^3$; r-sq =39.4% (Where X=PltTpGrn%120 (20)
$Y = -70.0 + 3.23X - 0.0437X^2 + 0.00019X^3$; r-sq = 15.0% (Where X=PltBtmGrn%120) (21)

$Y = -8048 + 155.6X - 1.000X^2 + 0.002138X^3$; r-sq = 16.4% (Where X= ErSenIni) (22)
$Y = -44180 + 834.8X - 5.255X)^2 + 0.01102X^3$; r-sq = 20.9% (Where X=ErSenCmp) (23)
$Y = 47.84 - 15.23X + 2.005X^2 - 0.08647X^3$; r-sq = 33.7% (Where X=50%ErSenDura) (24)

Correlation pattern of SG traits with grain yield
From bird's eye view, graphs show that there has been mostly weak correlation of grain yield with most of the SG traits of numbers of green leaves. But; from X-ray eyes' views, high and

low GYHs separately demonstrated strong polynomial correlation between grain yield and SG traits such as numbers of green leaves below ear (BtmGrn), number of leaves above ear (TopE0Grn) and total number of green leaves (GrnLvs) during entire GF. For example; TopE0Grn110, high GYHs 8, 12, 11, 13, 5 and 6 made a quadratic curve and low GYHs 10, 1, 14, 2, 9, 15, 4 and 3 have made different quadratic curve. In addition, high GYHs made highly responsive pattern between the trait and grain yield (Figure 1 and 2).

Similarly; in other curves constructed with other trait too; two types of patterns can be observed: one for medium to low GYHs and another for high GYHs (Fig 4.8.1-2). Here some hybrids can be cultivars and some cannot be. Here we can say; crop modelers work with cultivars. So, the pattern will be very useful to the crop modeler. Breeders too can utilize the pattern in association to other traits for selection breeding. In addition, days for 0% leaf green (or 100% leaf senescence) and 0% population green (or 100% population senescence) showed high pairwise correlation with grain yields among the most of the hybrids. So the trait of days for 100% population senescence can be breeder's valuable trait to select hybrid with high grain yielding superiority in hybrid maize breeding (Fig 1 & 2).

Although r^2 in polynomial regression equation constructed between grain yield and days for 50% green PP have been found higher than the days for 0% green of the hybrid PP; curve constructed between grain yield and days for 0% green PP (100% senescence of the PP) can depict substantial pairwise linear correlation (Fig 1 & 2).

DISCUSSION

Leaves just above ear and around ear node are more plant dry matter and GY determining than lower leaves below ear (He et al., 2003; Yan et al., 2011). Highest GYH 8 had medium to small numbers of Tp+E0 leaves, BtmGrn number and total numbers of green leaves, but it has been found still high grain yielding. Highest GY, short SG and medium to low numbers of green leaves imply that the H8 might have grain yield attributing highly photosynthetic leaves; but it lost green leaves faster than other high GYHs. In addition, there might be a pleiotropic positively grain yield contributing qualitative gene that might regulate faster leaf senescence to release soluble proteins for increasing GY in the hybrid (Fig 1 & 2).

High GYHs 12 and 13 had larger numbers of top green leaves that remained longer during entire GF than most of the high and medium grain yielding thirteen hybrids. It can be concluded that the hybrids 12 and 13 had top leaves of higher photosynthetic efficiency, highly protected against winter chilling and transitional period from winter to spring. In addition, the leaves of the hybrids 12 and 13 had higher photosynthetic leaves and for longer duration too which might be because of quantitative positively GY determining gene. Polynomial correlation curves constructed between GYs and numbers of total green leaves among the FHs on different dates during entire GF has also depicted two curves for two types of hybrids of high and medium to low GYHs (Fig 1 & 2).

Some medium to low GYHs 7, 14, 2, 9, 15, 4 and 3 have lower numbers of top green leaves and they lost the green leaves faster. So they were medium to low GYHs (Fig 1). It has been reported that photosynthesis declines if the maize plants lose SG prematurely (Tollenaar and Daynard, 1978; Wolfe et al., 1988a, b). The PPs of high GYHs 12, 11, 13, 5 and 6 lost SG very late in comparison to PPs of the low GYHs. The PPs of high GYHs lost SG two days late. This might be the reason of high GY in the high GYHs. Ma and Dwyer (1998) displayed evidence that maize hybrid with the trait of longer SG and longer GF after flowering produced 24% more dry matter and assimilated 20% more nitrogen than early SG losing hybrid during GF. Few exceptional low GYHs such as 1 and 10 too were parallel to high GYHs 6, 5, 11 and 13 in the 0% SG of the PPs. Why the two hybrids 1 and 10 were low grain yielding could not be explained in comparison to high GYHs 6, 5, 11 and 13 based on the analyzed tabulated data. Flowering behavior of the hybrids have been characterized in the same trial by Adhikari et al. (2015c).

Conventional maize breeders seek longer SG high GYHs since the trait of long SG contributes greatly to GY (Thomas and Howarth, 2000; Gan and Amasino, 1995; Borrell et al., 2000). Spano et al., (2003) displayed that elongation of GF boosts GY of crops and elongation is only possible if the trait of SG is improved delaying leaf senescence. Dynamics of SG leaves of the hybrids can be extracted from the Table 2. Temperature details during trial period from sowing to crop harvest have been summarized by Adhikari et al. (2015b).

Now, the concept of delaying SG should be a little bit changed. Since the crop of some high GYH cultivar becomes ready earlier for harvest since they have higher efficiency of photosynthesis and nutrient mobilization towards sink which can be extracted from the ST trait and GY of the highest GYH 8. For clarity, H 8 lost SG earliest among the FHs, but it was the highest grain yielding. Zhang et al. (2012) too displayed highly photosynthetic leaf lost green pigment earlier than longer SG trait. Thomas and Smart (1993); Thomas and Howarth (2000) also explained eight different types of rate of loss of SG and their effects on photosynthetic function.

In the trial; the highest GYH was 8, but it was the earliest maturing hybrid among the fifteen. But, second highest grain yielding hybrid 12 is very late maturing and SG stays longer than the hybrid 8. Some genotypes of maize is quick senescing but have traits of efficient and functional photosynthesis to contribute to GY; but some genotypes of maize is slow senescing and SG lasts longer, but low grain yielder with less efficient and less functional photosynthetic efficiency (Thomas and Howarth, 2000; Hörtensteiner, 2009). So the breeders should be oriented more towards high grain yielding with the trait of functional short SG with efficient photosynthetic efficiency instead of longer SG of low photosynthetic and poor functionality.

Supplementary file:

Adhikari NR, Ghimire SK, Sah SK, Koirala KB. (2015) Stay green traits of hybrids of maize (*Zea mays* L.) below and above ear during grain filling and their association with grain yields in winter in subtropical foot plain of Nepalese Himalaya.
DOI: 10.7287/peerj.preprints.920v1/supp-1
https://d3amtssd1tejdt.cloudfront.net/2015/920/1/Supplementary_file1.pdf

REFERENCES

Adhikari NR, Ghimire SK, Sah SK, Koirala KB. 2015a. Dynamics of chlorophyll and N concentration on e0 and e3 leaves of maize hybrids in winter in subtropical region. PeerJ PrePrints 3:e1050. http://dx.doi.org/10.7287/peerj.preprints.850v1

Adhikari NR, Ghimire SK, Sah SK, Koirala KB. 2015b. Details of chlorophyll and nitrogen concentrations of central and top two leaves of single cross hybrids of maize during grain filling, pedigree of the hybrids, climate of the growing duration in winter in subtropical foot plain of Himalaya. DOI:10.7287/peerj.preprints.850v1/supp-1 (https://d3amtssd1tejdt.cloudfront.net/2015/850/2/NRASupplemental_file_1.pdf)

Adhikari NR, Ghimire SK, Sah SK, Koirala KB. (2015c) Flowering, reproductive behaviors and their effects on grain yields of newly bred single cross hybrids of yellow maize (*Zea mays* L.) in winter in subtropical Nepalese Himalayan foot plain. PeerJ PrePrints 3:e1108 https://dx.doi.org/10.7287/peerj.preprints.897v1

Borrell AK, Hammer GL, Henzell RG. 2000. Does maintaining green leaf area in Sorghum improve yield under drought? II. Dry matter production and yield. Crop Science 40: 1037–1048.

Gan S, Amasino RM.1995. Inhibition of leaf senescence by autoregulated production of cytokinin. Science 270: 1986–1988.

He P, Osaki M, Takebe M, Shinano T. 2003. Comparison of whole system of carbon and nitrogen accumulation between two maize hybrids differing in leaf senescence. Photosynthetica 41:399–405.

Hörtensteiner S. 2009. Stay-green regulates chlorophyll and chlorophyll-binding protein degradation during senescence. Trends in Plant Science 14:155–162.

Leshem YY, Halevy AH, Frenkel C. 1986. Processes and Control of Plant Senescence, Elsevier Science Publisher BV, Amsterdaym, Netherlands. pp.250.

Ma BL, Dwyer LM. 1998. Nitrogen up take and use of two contrasting maize hybrids differing in leaf senescence. Plant and soil 199: 283–291.

Sexton R, Woolhouse HW. 1984. Senescence and abscission, in: Wilkins M.B. (Ed.) Advanced Plant Physiology, Longman Scientific and Technical, USA NY. pp. 469-497.

Spano G, Fonzo Di N, Perrotta C, Platani C, Ronga G. 2003. Physiological characterization of 'stay green' mutants in durum wheat. Journal of Experimental Botany 54:1415–1420.

Thomas H and Smart CM. 1993. Crops that stay green. Annals of Applied Biology 123:193-213.

Thomas H and Howarth CJ. 2000. Five ways to stay green. Journal of Experimental Botany. 51: 329-337.

Tollenaar M and Daynard TB. 1978. Leaf senescence in short-season maize hybrids. Canadian Journal of Plant Science 58: 869-874.

Wolfe DW, Henderson DW, Hsiao TC, Alvino A. 1988a. Interactive water and nitrogen effects on senescence of Maize.I. leaf area duration, nitrogen distribution, and yield. Agronomy Journal. 80: 859–864.

Wolfe DW, Henderson DW, Hsiao TC and Alvino A. 1988b. Interactive water and nitrogen effects on senescence on maize. II. Photosynthetic decline and longevity of individual leaves. Agronomy Journal 80:865-860.

Yan HF, Shang AX, Peng YF, Yu P, Li CJ. 2011. Covering middle leaves and ears reveals differential regulatory roles of vegetative and reproductive organs in root growth and nitrogen uptake in maize. Crop Science 51:265–272.

Zhang Z, Geng G, Gao H, Zhang L, Yang C, Liu P, Meng Q. 2012. Characterization of Photosynthetic Performance during Senescence in Stay-Green and Quick-Leaf-Senescence *Zea mays* L. Inbred Lines. PLoS One 7(8): e42936.

Table 1: Treatment details of the winter hybrid maize trial

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)**

Materials and methods of the trial and pedigrees of the above-mentioned hybrids have been mentioned in earlier paper (Adhikari et al. 2015a; b).

Table 2: Linear regression equations of dynamics of number of green leaves below and above the topmost ear have been extracted from fifteen randomly selected plants in three plots. Significance of the time series equations of dynamics of numbers of SG leaves below and above the topmost ear have been characterized by significance (*significant at 0.05 and ** very significant dependency between leaves and age at 0.01 level; NS denotes for non-significant dependency) and coefficient of determination (r^2).

Numbers of bottom green leaves (Yb)	Numbers of the (topmost) ear and above-ear green leaves (Yt)
(1)Yb = 20.0 - 0.112 Days (*) R-Sq = 0.91	Yt = 17.4 - 0.0823 Days (*) R-Sq = 78.5%
(2)Yb = 13.1 - 0.0745 Days (**) R-Sq = 0.95	Yt= 16.7 - 0.0809 Days (NS) R-Sq = 70.7%
(3)Yb = 16.6 - 0.0967 Days (**) R-Sq = 0.95	Yt= 16.2 - 0.0847 Days (*)R-Sq = 79.2%
(4)Yb = 13.6 - 0.0787 Days (**) R-Sq = 0.93	Yt = 16.4 - 0.0838 Days (NS) R-Sq = 77.0%
(5)Yb = 19.3 - 0.110 Days (**) R-Sq = 0.92	Yt = 20.1 - 0.102 Days (NS)R-Sq = 74.8%
(6)Yb = 15.1 - 0.0870 Days (*) R-Sq = 0.91	Yt = 16.7 - 0.0841 Days (NS) R-Sq = 72.5%
(7)Yb = 17.2 - 0.0992 Days (**) R-Sq = 0.93	Yt = 17.3 - 0.0927 Days (*) R-Sq = 81.9%
(8)Yb = 17.1 - 0.0993 Days (**) R-Sq = 0.96	Yt = 19.0 - 0.0993 Days (NS) R-Sq = 73.1%
(9)Yb = 17.5 - 0.0993 Days (*) R-Sq = 0.91	Yt = 19.3 - 0.0993 Days(NS) R-Sq = 71.3%
(10)Yb = 15.2 - 0.0843 Days (*) R-Sq = 0.91	Yt = 16.4 - 0.0782 Days(*) R-Sq = 80.6%
(11)Yb = 16.1 - 0.0883 Days (*) R-Sq = 0.85	Yt = 17.3 - 0.0811 Days (NS) R-Sq = 67.3%
12)Yb = 17.9 - 0.103 Days(**) R-Sq = 0.92	Yt = 15.2 - 0.0692 Days(NS) R-Sq = 69.3%
(13)Yb = 15.3 - 0.0867 Days (*) R-Sq = 0.90	Yt = 14.2 - 0.0613 Days (NS) R-Sq = 76.5%
(14)Yb = 18.6 - 0.105 Days (*) R-Sq = 0.90	Yt = 18.2 - 0.0942 Days (*) R-Sq = 78.1%
(15)Yb = 17.7 - 0.101 Days (**) R-Sq = 0.95	Yt = 16.3 - 0.0819 Days (NS) R-Sq = 77.0%

The hybrid entries included in the trial are (1) RML-19/NML-2 (1), (2) RL-137/RL-168 (2), (3) RML-55/RL-29(3), (4) RL-99/RL-161(4), (5) RML-6/RML-19 (5), (6) RL-111/RL-189 (6), (7) RML-95/RML-96 (7), (8) RML-86/RML-96(8), (9) RL-36/RL-197(9), (10) RL-180/RML-5(10), (11) RML-57/RML-6(11), (12) RL-170/RL-111 (12), (13) RL-154/RL-111(13), (14) RML-4/NML-2 (14) and (15) Gaurav (15).

Table 3: DMRT of days required for ear senescence traits of the single cross hybrids

Entry	Grain yield (t/ha)		ErSenIni		ErSenCmp		AllLfSene		Er Sen D	
			|-----------------------------days-----------------------------|							
8	12.54	A	150.0	B-E	156.5	BCD	165.7	C	6.5	CD
12	11.80	A	147.8	DEF	156.8	BCD	173.9	AB	9.0	A-D
11	11.55	A	151.8	A-D	161.1	AB	176.3	A	9.3	ABC
13	11.31	AB	147.7	DEF	156.6	BCD	174.3	AB	8.9	A-D
5	11.05	AB	152.7	ABC	160.6	AB	171.5	ABC	7.9	BCD
6	11.02	AB	145.2	FG	154.3	CD	172.1	ABC	9.1	ABC
10	9.78	ABC	152.3	ABC	160.6	AB	175.9	A	8.3	A-D
1	9.75	ABC	155.1	A	164.5	A	173.5	AB	9.4	ABC
7	9.70	ABC	149.7	B-E	157.1	BC	169.8	ABC	7.5	BCD
14	9.64	ABC	152.9	AB	158.7	BC	170.5	ABC	5.8	D
2	9.47	ABC	148.3	C-F	158.9	BC	170.7	ABC	10.5	AB
9	9.30	ABC	151.1	A-E	160.9	AB	169.0	BC	9.8	ABC
15	9.17	ABC	152.9	AB	159.7	ABC	172.3	ABC	6.8	CD
4	7.87	BC	143.1	G	151.7	D	171.1	ABC	8.6	A-D
3	7.03	C	147.0	EFG	158.3	BC	170.5	ABC	11.3	A
Mean	10.07		149.8		158.4		171.8		8.6	

Table 4: Mean and DMRT of days for stay green leaves and stay green hybrids

Entry	50%LfGrn	0%LfGrn		50%PopGrn		0%PopGrn	
8	155.7	174.3	C	166.3	D	175.3	D
12	153.7	178.0	AB	173.3	A	181.7	A
11	157.3	178.0	AB	171.0	ABC	179.0	ABC
13	154.0	178.3	A	173.0	AB	180.0	AB
5	156.0	176.3	ABC	169.7	ABCD	178.3	ABCD
6	152.3	176.3	ABC	171.3	ABC	178.3	ABCD
10	156.7	178.3	A	171.7	ABC	180.0	AB
1	153.7	176.7	ABC	170.0	ABCD	178.3	ABCD
7	153.3	174.3	C	169.3	BCD	175.7	CD
14	153.0	175.0	BC	169.7	ABCD	176.3	CD
2	153.7	175.3	ABC	169.0	CD	176.7	BCD
9	155.3	175.0	BC	170.0	ABCD	177.0	BCD
15	153.7	174.7	C	170.0	ABCD	176.7	BCD
4	154.0	174.0	C	169.7	ABCD	175.3	D
3	154.0	173.7	C	169.7	ABCD	175.0	D
Mean	154.4	175.9		170.2		177.6	

Table 5: DMRT of numbers of stay green leaves below ear of the maize hybrids

	|--------------------Numbers of bottom green leaves -------------------|								
Entries	110		125		140		155		170
8	5.7	A-D	4.97	B-E	3.93	CDE	1.53	B	0.00
12	5.8	A-D	5.43	ABC	4.43	A-D	1.47	B	0.07
11	5.5	B-E	5.30	A-D	5.03	AB	2.37	AB	0.37
13	5.0	CDE	4.87	B-E	4.07	B-E	1.40	B	0.23
5	6.4	AB	5.97	AB	4.90	ABC	2.23	AB	0.00
6	4.8	DE	4.63	CDE	3.73	DE	1.10	B	0.07
10	5.3	B-E	4.97	B-E	4.20	B-E	2.27	AB	0.30
1	6.8	A	6.47	A	5.27	A	3.03	A	0.13
7	5.7	A-D	5.27	A-D	4.17	B-E	1.53	B	0.10
14	6.1	ABC	6.00	AB	5.03	AB	2.03	AB	0.23
2	4.4	E	3.97	E	3.20	E	1.40	B	0.13
9	5.9	A-D	5.30	A-D	4.70	A-D	2.27	AB	0.00
15	6.1	ABC	5.37	ABC	4.40	A-D	1.87	AB	0.33
4	4.5	E	4.10	DE	3.30	E	1.07	B	0.08
3	5.5	B-E	4.93	B-E	3.70	DE	1.23	B	0.07
Mean	5.6		5.17		4.27		1.79		0.14

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), R -111/RL-189 (6), RML -95/RML-96 (7), RML -86/RML-96 (8), RL -36/RL -197 (9), RL -180/RML-5 (10), RM L-57/RML-6 (11), RL-170/RL -111 (12), RL -154/RL -111 (13), RML-4/NML -2 (14) and Gaurav (15).

Table 6: DMRT of numbers of stay green leaves above ear of the maize hybrids

Entry	TopEoGrn110		TopEoGrn125		TpE0Grn140		Tp+E0Grn155		Tp+E0Grn170	
8	6.87	BCD	6.83	ABC	6.80	AB	4.67	A-D	0.50	D
12	6.73	CDE	6.73	A-D	6.70	ABC	5.47	ABC	2.17	ABC
11	7.33	ABC	7.33	AB	7.27	A	6.03	A	1.90	A-D
13	6.73	CDE	6.70	BCD	6.70	ABC	5.10	A-D	2.93	A
5	7.80	A	7.43	A	7.33	A	5.57	AB	1.10	BCD
6	6.40	DEF	6.30	C-F	6.27	B-E	4.63	A-D	0.93	BCD
10	7.07	BC	6.63	B-E	6.60	A-D	4.90	A-D	2.07	A-D
1	7.47	AB	7.32	AB	7.12	A	5.37	ABC	2.27	AB
7	6.13	EF	5.97	EF	5.87	DE	3.07	E	0.63	BCD
14	6.73	CDE	6.67	B-E	6.67	ABC	4.00	CDE	1.00	BCD
2	6.87	BCD	6.73	A-D	6.60	A-D	5.40	ABC	1.47	A-D
9	7.27	ABC	6.93	ABC	6.90	AB	5.37	ABC	0.60	CD
15	6.27	DEF	6.23	C-F	6.23	B-E	4.03	B-E	1.23	BCD
4	6.20	EF	6.07	DEF	5.97	CDE	4.10	B-E	0.90	BCD
3	6.00	F	5.83	F	5.73	E	3.67	DE	0.73	BCD
Mean	6.79		6.65		6.58		4.76		1.36	

Table 7: Mean and DMRT of stay green and senescence traits of the hybrids

	|------Numbers of total green leaves at different time during grain filling------|									
Entries	110		125		140		155		170	
8	12.60	CD	11.80	C-F	10.73	BCD	6.20	A-E	0.50	C
12	12.57	CD	12.17	B-E	11.13	ABC	6.93	A-E	2.23	ABC
11	12.87	C	12.63	ABC	12.30	A	8.40	A	2.27	ABC
13	11.73	D-G	11.57	C-F	10.77	BCD	6.50	A-E	3.17	A
5	14.17	AB	13.40	AB	12.23	A	7.80	AB	1.10	BC
6	11.23	GH	10.93	EFG	10.00	C-F	5.73	B-E	1.00	BC
10	12.33	C-F	11.60	C-F	10.80	BCD	7.17	A-D	2.37	AB
1	14.27	A	13.78	A	12.38	A	8.40	A	2.40	AB
7	11.80	D-G	11.23	D-G	10.03	C-F	4.60	E	0.73	BC
14	12.87	C	12.67	ABC	11.70	AB	6.03	A-E	1.23	BC
2	11.30	FGH	10.70	FG	9.80	DEF	6.80	A-E	1.60	ABC
9	13.20	BC	12.23	BCD	11.60	AB	7.63	ABC	0.60	BC
15	12.40	CDE	11.60	C-F	10.63	B-E	5.90	A-E	1.57	ABC
4	10.67	H	10.17	G	9.27	F	5.17	CDE	0.98	BC
3	11.47	E-H	10.77	FG	9.43	EF	4.90	DE	0.80	BC
Mean	12.36		11.82		10.85		6.54		1.50	

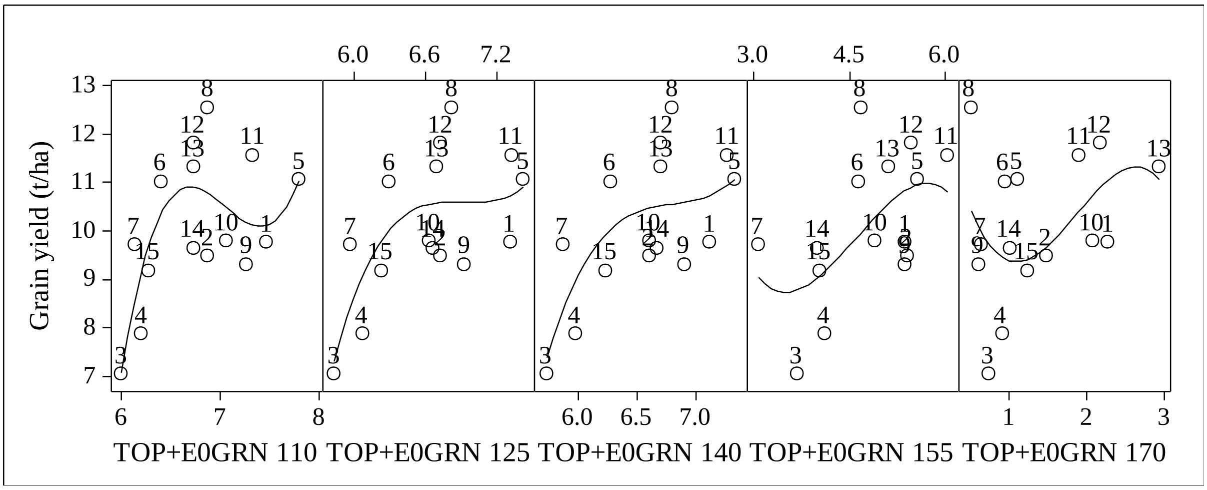

Fig 1: Association curve between above-ear green leaves numbers and grain yields. The curves are drawn for the fifteen hybrids. How the newly bred hybrids behave in the trial in response to above-ear green leaves during grain filling can be extracted from the curve. (A) TOP+E0GRN indicates numbers of green leaves above ear including e0 leaf. Leaf accompanied by the number indicates days after sowing when the data were recorded or days after sowing (DAS).

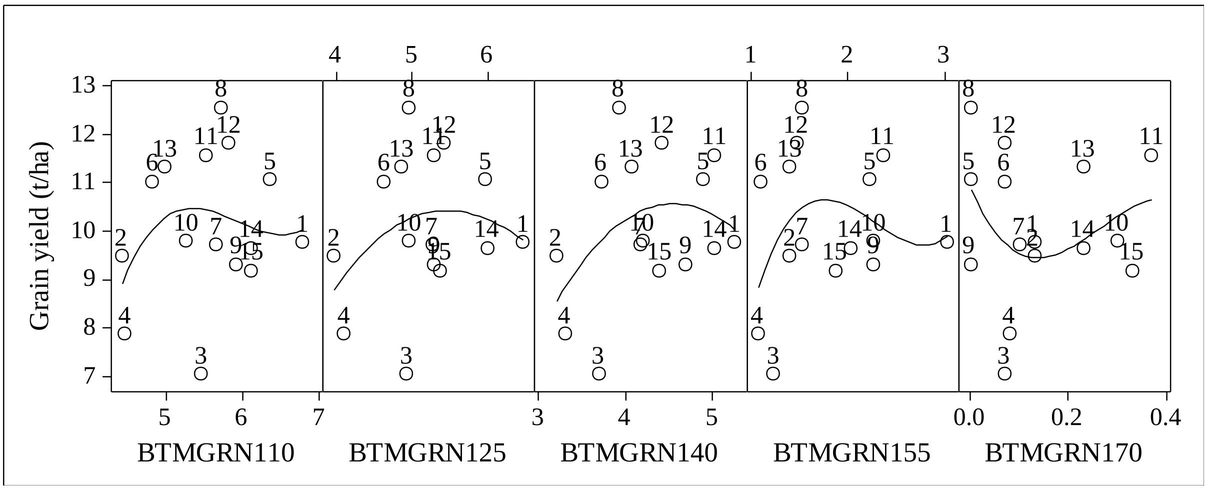

Fig 2: Association curve between grain yields and numbers of below-ear green leaves. The curves are drawn for the fifteen hybrids. Hybrid entries are included in the trial A1 are RML-19/NML-2(1), RL-137/RL-168(2), RML-55/RL-29(3), RL-99/RL-161(4), RML-6/RML-19(5), RL-111/RL-189(6), RML-95/RML-96(7), 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).

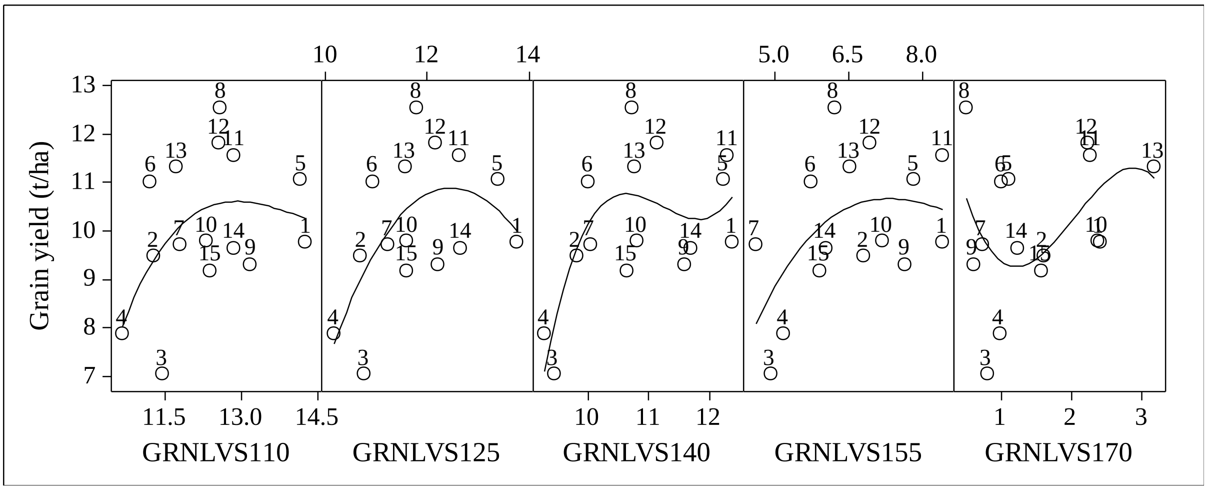

Fig 3: Association curve between grain yields and numbers of total green leaves. The curves are drawn for the fifteen hybrids.

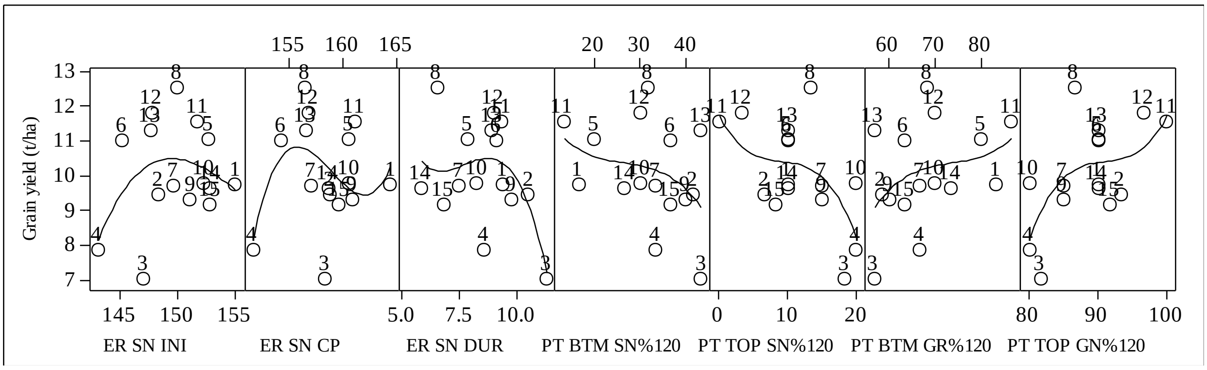

Fig 4: Association curve between grain yields and senescence traits of the hybrids. The senescence traits are days for ear senescence initiation (Er SN INI), ear senescence completion (ER SN CP), net ear senescence duration (ER SN DUR), percent senescence below ear in the plots (PT BT MSN%120), percent senescence above ear in the plots (PT TOP SN%120), percent greenness below ear in the plot (PT BTM GR%120), percent greenness above ear (PT TOP GN%120) on 120th day after sowing.

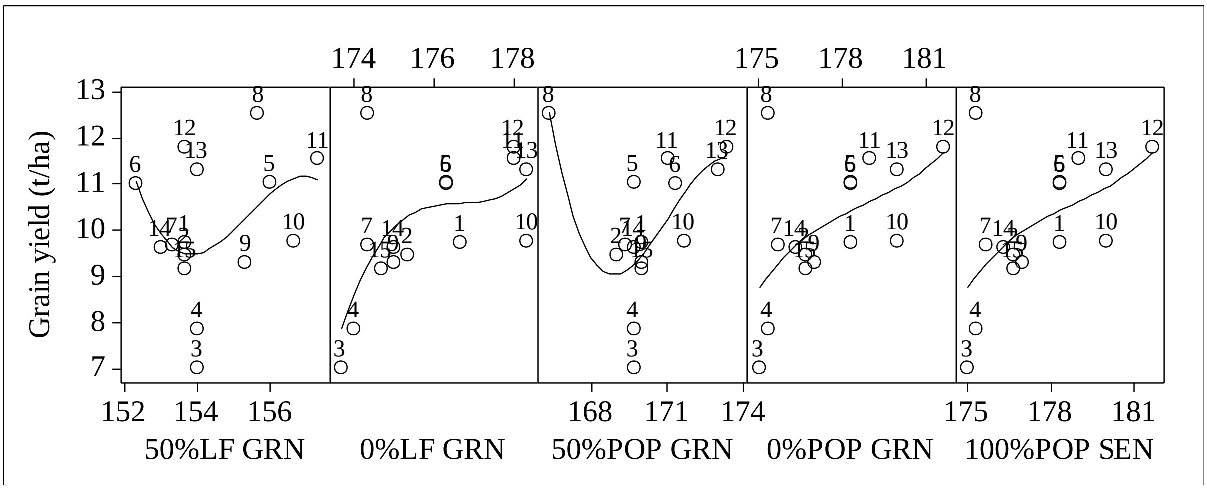

Fig 5: Association curve between grain yields and senescence traits The curves are drawn for the fifteen hybrids. The senescence traits are days for remaining 50% green leaves in plot (50% LF GRN), days for 0% green leaves in the plot (0% LF GRN), days for 50% green HPP (hybrid plant population) in the plots (50% POP GRN, days for 0% green HPP (0%POP GRN) and days for 100% senescence of the HPP (100%POP SEN) of the fifteen populations. Grain yield t/ha = - 516303 + 8768*(days for 0%LFGRN) - 49.63*(days for 0%LFGRN)^2 + 0.0936* (days for 0%LFGRN)^3, R-Sq = 41.1%.