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

Floral traits: grain yield determinants

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ABSTRACT

Introduction: All the plants of a maize population or hybrid PP (plant population) do not dehisce anthers and do not emerge silk simultaneously. Generally, male flowering or anther dehiscence (anthesis) is leading and female flowering (silk emergence or silking) is lagging under both conducive and stressful environment. Enough and timely pollen availability is indispensible when silks come out of maize ears for largest number of kernel set. So; study of flowering and reproductive behavior of newly bred maize hybrids are indispensible to confirm high grain yielding hybrid with trait of simultaneous anthesis and silking (synchrony). **Materials and methods:** Flowering and reproductive behaviors of fifteen newly bred single cross hybrids of yellow maize have been examined in winter growing them in an RCBD trial planting their seeds on October 3, 2012 in subtropical foot plain of Himalaya. For flowering (FB); emergence of male organ (emergence of tassel or tasseling or TSS) from apical node of the stem, anthesis, silking, browning of silk or withering of silk (silk senescence or S SEN), tassel-anthesis interval (TAI), anthesis-silking interval (ASI), silking-silk senescence interval (S INI-S SEN Interval) of the hybrids have been examined dissecting the plant population (PP) into four equal parts (percent) as the first earliest, second earliest, third earliest and terminal 25% of the PP denominated as 25, 50, 75 and 100% respectively of the each of the fifteen hybrids. So, whether third earliest 25% and terminal 25% silk emerging PP denominated as SILK 75 and SILK 100% respectively will get enough pollen from the PP of the same hybrid can be determined through ASI 75 and ASI 100 of the hybrid PP. **Results and discussion**: A polynomial grain yield estimating regression equation (t ha^{-1}) Y = - 11922 + 507.3*(TSS25) - 7.183*(TSS25)^2 + 0.03387*(TSS25)^3 with r^2 value 66% has been discovered from days taken for TSS25 (TSS of earliest 25% PP). Similarly, high r^2 bearing polynomial equations were from days for anthesis and silking of the PPs of the fifteen hybrids. Equations with the lowest r^2 were for the days required for the silk senescence (S SEN). Reason of low r^2 is that the observation of silk browning or withering could not be precise for naked eye since the silks looked fresh longer in high humidity, low temperature and cloudy day in the winter although it lost receptivity earlier. In addition, Interval duration from S INI to S SEN cannot be the duration of silk receptivity in the winter of subtropical Nepalese Himalayan foot plain. For reproductive behavior; number of functional egg cells that became successful zygotes to pull assimilates for complete kernel development can reflect aroma about reproductive strength (RS) of the hybrid. Accordingly, hybrids 8 and 5 have been found of the highest RS.

INTRODUCTION

Almost all food grains we consume come from crops after sexual fusion of the male and female gametes from male and female floral organs. Male gamete, its generating organ (Herrero, 2003; Hedhly et al., 2008) and microsporogenesis phenomena (Oliver et al., 2005) are more sensitive to chilling and cold temperature than egg cell and its formation phenomena. Most crop producing region in the world has been facing global climate change associated extreme weather fluctuation (Solomon et al., 2007). In other words, productivity and global production of most of the crops including maize in most of agricultural land in the world is at the mercy of natural climate and fluctuating environmental abiotic factors. All cereal production takes place through the results of the phenomena of crop growth and development, and grain filling.

Male and flowers both are located in maize plant (monoecious); but they bear in different sites in the plant. Flowering, preparation of flowering and reproduction (Harris et al., 1976; Motto and Moll, 1983; Sarquis et al., 1998; Freier et al., 1984; Carcova et al., 2000) are very are very sensitive to extreme climatic factors. It is because chilling and very high N dose application damage rice crop (Tatsuta, 1999; Hayashi et al., 2000; Sasaki and Wada, 1975; Amano and Moriwaki, 1984; Satake et al., 1987). Fertilization of high dose of nitrogen is required for optimal growth of the rice crop and to increase the grain production (Shiga, 1984; Goto et al., 2006; Fukushima, 2007). Nishiyama (1982) also reported that number of pollen grains per anther is highly correlated with soil fertility. But, excessive N fertilization causes reduction in floral potency which was declared through decline in the number of pollen grains (Tatsuta, 1999; Hayashi et al., 2000). The pollen germination ratio was similarly found declined further when high dose of nitrogen fertilization was done under the cool weather temperature during flowering which was indicated from declined number of engorged pollen grains (Hayashi et al., 2006). It has been reported that less fertile spikelets were observed in rice crop grown in cool temperature during flowering with high nitrogen fertilization (Sasaki and Wada, 1975; Amano and Moriwaki, 1984; Satake et al., 1987). As the result, rice production declined. The phenomena too can be homologous in maize since they are both tropical crop species. Crop damage because of any suboptimal temperature in any one of the three vital phenomena: pollen production, pollination and fertilization alone or in combination cause heavy reduction in grain yield of crops (Oliver et al., 2005; Sataka and Hayase, 1970; Hayase et al., 1969; Sataka and Hayase, 1970).

Cool temperature causes floral impotency lowering the number of engorged pollen grains during pollen formation in the rice. Satake and Shibata (1992) reported that successful fusion of male gamete to egg cell is only possible if the stigma receives at least 40 engorged pollen grains under cool weather during flowering to fertilization. Extreme chilling and cold temperatures can induce dysfunctioning of pollens causing sterility because of disruption of starch metabolism in anther tapetum which ultimately stops starch accumulation in the pollen grains in rice (Oliver et al., 2005). Cold induced disruption of sugar transport into anther and its corresponding detrimental effects on pollen causes sterility which is signaled by ABA and turning off of the expression of cell wall invertase (Oliver et al., 2005) and monosaccharide transporters (Oliver et al., 2007). Extreme chilling stress can impair microsporogenesis leading to prevention of the maturity of microspores into trinucleate pollen grains (Sataka and Hayase, 1970) in the rice. Cool temperature in summer growing season has been causing a serious decline in rice production because of failure in the pollen germination and gamete fusion.

Chilling temperature is very fatal to maize during flowering, preparation of flowering or reproductive phase in winter in subtropical region is fatal for formation, vitality, germination of the pollens, its efficiency to penetrate ovary wall and reach embryo sac for egg cell fertilization. Such aberrant phenomena lead subsequent irreversible grain yield loss. Prevalence of extreme chilling temperature at any phase from pollen formation to its utilization for zygote formation in the maize in the winter in Nepal and north eastern Indian region or in subtropical region is serious stress factor to cause complete failure of the crop which has been mentioned in introduction of the paper of Zaidi et al. (2010). So, synchrony in the phenomena of anthesis and silking (Harris et al., 1976; Motto and Moll, 1983; Sarquis´s et al., 1998; Freier et al., 1984); synchronous coming out of all silks, synchronous pollination of all silks and synchronous fertilization of egg cells in all florets are very indispensable for highest number of kernel set and grain yield attribution (Carcova et al., 2000) in response to extreme chilling.

Maize breeders seek hybrids and their paired inbreds with minimum anthesis-silking interval (ASI) for success of hybrids. The narrow ASI is (anthesis-silking interval) very useful trait to increase kernel numbers in maize genotype(s) (Bolanos and Edmeades, 1996; Ribaut et al., 1996) in addition to other morpho-physiological traits. So, ASI of each hybrid must be examined minutely. Grain yield is positively and strongly correlated to kernel numbers (Tollenaar et al., 2000; Echarte et al., 2000). Edmeades and Daynard (1979) first time reported that how many kernel will set in an ear of the maize plant is positively correlated to the rate of photosynthesis per plant during anthesis. The equation extracted from their experiment has been used to predict kernel number per plant and then incorporated into the original version of CERES-Maize (Jones and Kiniry, 1986). It has been reported that prevailing temperature, photoperiod and synchrony between anther dehiscence and silking (SAI) are ear kernel number determining traits (Struik et al., 1986) in addition to plant photo-assimilate supply (Edmeades and Daynard, 1979). Temperature and photoperiod like environmental and physiological factors affect the floral development eventually influencing the kernel number determination in the maize ears (Herrero and Johnson, 1981; Hall et al., 1982; Struik et al., 1986; Bassetti and Westgate, 1993a).

There have been some reports of advanced studies of genetic architecture of flowering of maize. Flowering of maize as a crop species have been done and reported. But, a new method must be explored to examine flowering behavior which helps do minute study of those newly bred hybrids and genotypes which are in the process of cultivar release. So, the paper includes the development of a new methodology and its illustration to examine flowering of any newly developed lines. Minute studies of flowering behavior of newly bred hybrids have been done using the new method to determine whether (a) particular hybrid(s) can be advanced for cultivar release. Flowering and reproductive traits of the newly bred hybrids have been minutely studied dissecting the plant population of each hybrid in each plot into four fractions since flowering and reproductive traits determine grain yield as well as fate of the maize hybrids and abnormal expression of the traits causes irreversible crop failure. The paper also includes results of a variety of flowering and reproductive traits of the fifteen hybrids and discovery of grain yield estimating equations from the traits.

MATERIAL AND METHODS

Trial accomplished, treatment details

In order to examine flowering and reproductive behaviors of the newly bred maize hybrids in subtropical Nepalese Himalayan foot plain; an experiment was conducted in RCBD on October 3, 2012 in research field of NMRP/NARC at Rampur (longitude $27^0$37'N, latitude $84^0$24'E and altitude 228 m above sea level), Chitwan, Nepal. Seeds were manually planted using a simple lever of seed drill. Two seeds were dropped in each hill in spacing of 0.25 m x 0.70 m and each two-row-plot size was 1.4 m x 3.0 m. Plants were thinned on 30 days after sowing (DAS) to maintain PP at the rate of 57,143 ha^{-1}. Plots were continuous and borders were planted with newly released cultivar: Rampur hybrid 2. Plots were made free from weeds and earthing-up was done on 45 DAS. Treatment details as the fifteen newly bred single cross hybrids of the yellow maize for the trial have been mentioned in Table 1. Pedigrees of the hybrids and summary of climate of the crop plants of the trial period have been shown by Adhikari et al. (2015b) and details research methods, planting, intercultural operations have been mentioned by Adhikari et al. (2015a).

Traits of flowering and reproductive behaviors (FB) examined using a new method

Traits of floral, flowering behavior of the hybrid maize included in the research work are days for tasseling (Table 4), anthesis (Table 5), TAI (tasseling-anthesis interval) (Table 4), silking (Table 6), ASI (anthesis-silking interval) (Table 7), silk senescence (Table 8), S INI-S SEN (interval duration from silk initiation or emergence from ear to silk senescence (Table 9). The silk senescence was recorded days for withering or browning of the silks. For detail analysis of flowering, HPPs have been dissected into four equal dissects or percent such as 25, 50, 75 and 100% HPP for tasseling (Table 4), anthesis (Table 5), silking (Table 6), ASI (Table 7), silk senescence (S SEN) (Table 8), silk senescence-silk initiation interval (SILK SEN-S INI) of HPP (Table 9). In addition; ear versus cob length, total versus significant ears per plant, average row numbers, average numbers of kernels (AV KNS) in each row, AV KNS per topmost ear and in each plant have also been examined (Supporting file, Sup Table 2).

Tasseling and anthesis (TAI)

Days for tassel emergence of randomly selected five plants in each plot, days for tassel emergence of first 25, second earliest 25, third earliest and terminal 25% PP or 25, 50, 75 and 100% PP (abbreviated by TSS, TSS25, TSS50, TSS75 and TSS100 respectively) have been studied taking their observation from each of 45 plots (Table 4). Net tassel emergence duration is difference between days for tassel emergence of 100% and 25% PP (TSS100-TSS25). Data were recorded from five randomly selected plants and plot performance both. Anthesis 25 is meant for days for first earliest 25% PP, anthesis 50 is meant for days for anthesis of second earliest 25% population or earliest 50% PP, anthesis 75 is meant for days for anthesis of third earliest 25% HPP or terminal 50% and anthesis 100 is meant for days for anthesis of terminal 25% PP or days for completion of the entire HPP. The traits are abbreviated as ANTH 5, ANTH25, ANTH50, ANTH75, and ANTH100 respectively). In addition, days for anthesis of randomly selected five plants in each plot was also observed and examined. Net anthesis duration as difference between days for anthesis of 100% and 25% population (ANTH100-25) have also been examined (Table 5). In the same way; TAI5 (tasseling-anthesis interval) of randomly selected five plants in each plot was also recorded and analyzed. In addition; 25, 50, 75 and 100% PP (abbreviated by ANTH-TSS25, ANTH-TSS50, ANTH-TSS75, and ANTH-TSS100 respectively or by TAI, TAI 25, TAI50, TAI75 and TAI 100) were also recorded and analyzed (Table 4).

Anthesis, silking and silk senescence

In the same way; days taken for silking of 25, 50, 75 and 100% HPP (abbreviated as SILK 25, SILK 50, SILK 75 and SILK 100 for for silking of the earliest 25, second 25, third 25 and terminal 25% PP respectively) were recorded and analyzed. Silking of five randomly selected plants in each plot were also recorded as abbreviated as SILK5 (Table 6). Similarly, the ASI has been dissected as ASI 25, ASI 50, ASI 75 and ASI 100 for the earliest 25, second 25, third 25 and terminal 25% PP (Table 7). In the same way, silk senescence has been dissected into four equal parts of the HPP (Table 8). In the same way, silk senescence (S SEN or s sen), silk sen-s ini (S SEN-S INI) has been dissected into four fractions of the HPP (Table 9).

Active silking duration has been determined as the interval from initiation to termination of silking in HPP. Active silking duration is duration from days for silking of 100% and 25% PP (SILK100-25) (Table 6). Days for silk senescence of the five sampled plants, earliest 25, 50, 75 and 100% PP (abbreviated by SILKSN 5, SIlKSN25, SIlKSN50, SIlKSN75, and SILKSN100 respectively) are days required for silk withering or browning for the earliest 25, second 25, third 25 and terminal 25% PP. Active silk senescence duration as duration from days for silk senescence of 25% to 100% PP (abbreviated as SILKSN100-25) (Table 8). Silk-senescence-silk initiation interval of randomly selected five plants in each plot, 25, 50, 75 and 100% PP (abbreviated by S SN-S INI 5, S SN-S INI25, S SN-S INI50, S SN-S INI75, S SN-S INI100) were also computed and analyzed (Table 9).

Floral details

For floral details; values of traits considered for the study are ear length in cm (Ear length), total ears in 100 plants (Tot Ear Nos), significant ear numbers in 100 plants (Sign ears), number of rows in topmost ear (E1Rws), average number of kernels in a row of topmost ear (E1Knl/row), length of the topmost ear in cm (Ear1Len), length of the cob of the topmost ear in cm (CobLen1), percent of cob length in cm to ear length in cm [Cob L/Ear L] (Supporting file, Sup Table 1).

Similarly, data of days for senescence of 100% population was taken under the heading of PopSen100 for crop maturity days. Information of total ears per plant (abbreviated by Tot ers/plant, functional ears per plant or significant ears per plant (denoted by F-ears/plant or sig ears /plant) were also computed taking data of total ears and significant ears in the five randomly selected plants in each plot during terminal crop maturity and transferred into tot ears per plant and significant or functional ears per plant. For observation of grain yield attributing characters, ear length, cob length for top most ear were measured when standing plants in plots went into crop maturity stage (Supporting file, Sup Table 1).

Just before the harvesting of the crop; row number, kernels per row, kernels in each topmost row determined and total kernel numbers per plant were determined. Then plant average for each plot was used for variance analysis and DMRT computation. These traits reflect reproductive strength (Table 7). The reproductive strength (RS) is measured through average total number of functional egg cells in ears of the randomly selected plants in each plot or hybrid that become successful zygotes to attract photo-assimilates and develop into complete kernel in a plant (TotKnls) for each hybrid. Data analysis of the traits has been done using computer software: Microsoft Excel, MSTAT-C, Minitab and Genestat.

RESULTS

Variance analysis: Variance analysis has been done for five types of anthesis: earliest 25% population designated as earliest 25%, second earliest 25% designated as 50%, third earliest 25% anthesis designated as 75%, terminally dissected 25% population designated as 100% and anthesis for five sampled plants for each plot (anthesis). The fifteen hybrids have been found non-significant for active anthesis period which is the period from start of anthesis of earliest 25% to the terminal 25% dissected population. The maize hybrids were significant different from the standpoint of days for tassel emergence (Supplementary file, Sup Table 1).

The hybrids were significant different from the standpoint of silk duration among five sampled plants, 25, 50, 75 and 100% of the PP including the active silk duration (ACT SILK DURA). The hybrids were also significant different from the perspective of the five traits of the ASI. In addition, the hybrids were significant different for each of four equal 25% fractions of the entire HPP for silk senescence. But the hybrids were non-significant different from the standpoint of the active silk senescence duration (ACT S SEN D). Here, stay-active silk is used instead of stay-receptive silk since exact receptiveness of the silk cannot be precisely based on silk senescence since it took many days for initiation of dark browning of the silk in the chilling winter in the subtropical region in Nepal. Hybrids were significant different to other floral and reproductive traits: average ear nos per plant (AV ER NOS P^{-1}), significant ear numbers (nos) per plant, nos of kernels in a row in e1 ear, average number of kernels set (AV KNS) in the first ear, average total number of kernels set (AV KNS) in a plant, length of ear 1, length of the cob 1, percent ear length for the cob, days for ear senescence initiation (ER SEN INI), ear senescence completion (ER SEN CMP) and ear senescence duration (ER SEN D (Supplementary file, Sup Table 1).

Evaluation of the hybrids: Six hybrids **(**Hs) 8, 12, 11, 13, 5 and 6 have been found the top highest grain yielding (HGYHs) among the fifteen. Among them, all four fractions of H 13 had delayed 6 days for expressivity of anthesis even after tassel emergence. It implies that the hybrid 13 were aberrant in male flowering. In addition, the hybrid cannot be advanced for further evaluation and cultivar release. Remaining five hybrids in all the four fractions of the PP did not delay in expressivity of anthesis after tassel emergence. HGYH 8 (RML 86/RML96) is characterized with the traits of anthesis-silking interval (ASI) 25, 50, 70 and 100 with 0, 2, 3 and 10 days respectively, active anthesis duration 6 days which is relatively short among the fifteen hybrids and active silk duration 16 days which is long. HGYH 8 is characterized with the traits of higher numbers of ears per plant, highest numbers of significant ears. H6 too had somewhat wide ASI in all four fractions of the PP. Anthesis, silking and silk senescence details have been presented in Tables 4, 5, 5, 6 and 7.

Seven hybrids 10, 1, 7, 14, 2, 9 and 15 were intermediate grain yielding. Among them, all four fractions of H 10 delayed 6 days for expressivity of anthesis even after tassel emergence. It implies that the hybrid 10 was aberrant in male flowering. In addition, the hybrid cannot be advanced for further evaluation and cultivar release. Remaining six hybrids in all the four fractions of the PP did not delay expressivity of anthesis after tassel emergence in majority of the hybrid PPs. Medium GYHs 1 and 9 is characterized with the traits of ASI 25, 50, 70 and 100 with wide among the fifteen hybrids. Among the seven; H 2 demonstrated wide interval between expressivities of tassel emergence and anthesis. Hs 4 and 3 were low grain yielding (Table 4, 5, 6, 7, 8).

Reproductive strength (RS) of the hybrids

RS of the hybrids is indicated through functional egg cell that that becomes successful to attract male gametes from the dividing generative nucleus and attract photo-assimilates from leaves and stem reserve to develop functionally into complete kernels with endosperm and embryo in maize. Average number of kernels set (AV KNS) per plant can reflect functional RS of the hybrid. Among the HGYHs, Hs 8 and 5 were the highest kernels bearing two among the fifteen. Top HGYH 8 expressed silking 0, 2, 3 and 10 days late in the first, second, third earliest and terminal 25% PP in comparison to anthesis in the HPP. It means that terminal 25% late silking HPP could not receive pollens from the same HPP. Two ears in combination of the H 8 produced highest numbers of kernels. It implies that lower ear of the terminal 25% the HPP received pollens from surrounding next hybrid. Although H 5 was of the second highest reproductive strength since it was the largest AV KNS bearing among the fifteen hybrids, it demonstrated wide expressivity of silking in the terminal 50% PP. It also implies that terminal 50% late silking PP could not receive enough engorged pollens from the same PP. Fifth HGYH 5 too emerged silks 6 and 8 days late in comparison to anthesis in the terminal 50% PP. It also implies that kernels set in the terminal 50% the HPP received pollens less from the same HPP; but received much pollen from the surrounding next HPP. So, the HGYHs 8 and 7 demonstrated asynchrony in male and female flowering. So, the two hybrids cannot be advanced ahead for cultivar release.

Reproductive strength of maize cultivars, hybrids and populations can be reflected through average total numbers of kernels (AV KNS) per plant, AV KNS of the topmost ear. Number of filled kernels in a single row and number of rows can also reflect the reproductive strength of the hybrids. Prolificacy of the hybrids are reflected through average total number of ears (AV ER N per plant and significant AV ER N that is filled with kernels. In supporting file, the information of the fifteen hybrids has been shown.

Floral traits as grain yield estimator

Days required for tasseling gave higher or equal r square polynomial equations (Eq 1-5) when compared with the r-square values of equation obtained by days required for anthesis (Eq 6-11). Equations discovered from the days required for silking (Eq 12-16) were less reliable than equations obtained from days for anthesis since the r-square values of the Eq 12-16 were less than Eq 6-11 obtained from anthesis. Least reliable polynomial regression equations were for days for silk senescence of the HPP than the equations obtained from silking. Climate data from tassel emergence to silk senescence clearly shows the climate of the duration was with suboptimal chilling evening to morning and low temperature in day time mentioned by Adhikari et al. (2015b). In addition, the days from tasseling to silk senescence were foggy and cloudy and the sunshine did not dry the silks up to the midday. Sunshine hours was not recorded and the sunshine hours in each day were almost less than four hours with low temperature in each day from 65 to 105th day after sowing (Personal memorization; Adhikari et al., 2015b). Although the silk lost receptivity earlier, the silk freshness up to longer duration made confusion in recording silk receptivity. The net duration or interval duration from silk emergence to silk senescence could not reflect silk receptivity. So, the polynomial equations discovered from the days required for silk senescence of the HPP had lower r-square than tasseling, anthesis and silking. Polynomial correlations of grain yields and kernels number per plant of each of the fifteen hybrids with anthesis and silk senescence have been shown through graph (Fig 1).

Days required for anthesis of five sampled plants in each plot, first, second, third and terminal 25% population of the fifteen hybrids could produce grain yield in t ha^{-1} through the polynomial correlation equation with coefficient of determination from 44 to 58%. Highest R-square was 58% for grain yield estimating equation from ANTH100. Here, ANTH100 is meant for days required for anthesis of the terminal 25% PP of the hybrids. In the correlation curve, hybrid 8 has been found highest grain yielding that could not come to the correlation curve (Fig 4.4.1). Besides; the hybrid 8 lowered r-square (Table 4.4.1). Data of average days required for silking, average days required for silk senescence of five randomly selected plants in each plot of the fifteen hybrids too could estimate grain yield regression equation with r-square 26 to 46%. Here; a variety of equations extracted from floral traits and average kernels numbers per plants of the hybrids have lower coefficient of determination (r-square) than equations for estimating grain yield in t ha^{-1}.

DISCUSSION

Why study of flowering and reproductive behavior? All the plants in the same hybrid population grown in the same date are not uniform in expressivity in the traits of anthesis and silking because of micro-variation in bulk density, soil moisture content, nutrient level and other soil factors. Anthers of certain percent of the PP dehisces pollens earlier than the certain percent of the earliest silk emerging PP. There is lagging of silk emergence in comparison to anther dehiscence. Terminal percent of late silk emerging population may not receive pollen from the same population. So, minute analysis of flowering must be done from earlier step of hybrid evaluation to the final. Fate of the terminal 25% late silk emerging population of each hybrid must be determined during evaluation. Furthermore; in global warming associated temperature fluctuation and longer duration of extreme chilling temperature (Solomon et al., 2007) can cause flowering and reproductive abnormality (Oliver et al., 2005) leading to pandemic crop sterility and heavy yield loss (Zaidi et al., 2010).

Plant breeders create variation, they identify useful variants, they exploit variation and they again identify superior variants for high grain yielding with quality traits and tolerance / resistance to biotic and abiotic stresses. Here; the single cross yellow maize hybrids developed include inferiors as well as superiors both. Plant trait measure as X variable have been found grain yield and kernel numbers determining through the use of discovered polynomial regression equations; but the discovered equations are of low r-square.

Grain yield (t /ha) estimating equations have been shown from equations 1 to 21 (Table 1). High grain yielding hybrids convert light energy, CO2 and the soil nutrients into photo-assimilates in the form of kernels efficiently because of high efficiencies in CO_2 fixation, phloem-loading and unloading, reserve mobilization, flowering and reproductive fitness, and tolerance to a variety of biotic and abiotic stresses. But, inferiors work less efficiently in any one or some of the physiologies.

Moreover, the efficiently fixed CO_2 does not mobilize to kernels uniformly in all the hybrids. So the equations extracted from the two variables ignored so many physiologies of aberrant or optimal kind. That is why the coefficient of determinations (r-square) of the grain yield as well as kernel numbers estimating equations is low. For example, efficiently or optimally flowering and reproductive hybrids may have poor or high efficiency of reserve mobilization and susceptible or tolerance to winter chilling during grain filling. These two types of hybrids violate the correlation pattern and subsequently discovered equations estimates output poorly with low r-square. Another example; optimal flowering and reproductive hybrids that have high efficiency of reserve mobilization but minimum chlorophyll concentration can also yield high (Adhikari et al., 2015a; b).

Reproductive phenomena of rice in response to application of high dose of N fertilizers and suboptimal natural chilling can be homologous to maize reproductive phenomena since both crop species are tropical origin. Excessive nitrogen fertilization caused decline in floral potency which was declared through decline in the number of pollen grains (Tatsuta, 1999; Hayashi et al., 2000). Hayase et al (1969); Satake and Hayase (1970) reported that rice microspores is very sensitive to cool temperature during flowering phase. Satake (1991) reported that cool temperature causes reduction in the number of microspore and pollen grains during microsporogenesis. The pollen germination ratio was similarly found declined further when nitrogen fertilization was done under the cool weather temperature during flowering (Hayashi et al., 2006) because of declined number of engorged pollen grains. It has been reported that less fertile spikelets were observed in rice grown in cool weather during flowering with high nitrogen fertilization (Sasaki and Wada, 1975; Amano and Moriwaki, 1984; Satake et al., 1987). As the result, rice production declined.

Hayashi et al. (2009) reported that the combined effect of high nitrogen application and cool weather caused repression of many genes than the expressed genes under cool temperature which consequently caused lower pollen germination on stigma in the rice plants in high N combined with cool temperature. In crop species of rice, 50 to 150 pollens were observed on each stigma and fertility of those plants was over 95% in optimal temperature; but, 50 to 100 pollens were observed in the stigma and corresponding plants had 50 to 80% fertility (Hayashi et al., 2004). They further reported that cooling weather causes reduction in pollen production and high nitrogen condition caused more cooling damage dysfunctioning the rice pollens.

Maize genetic resources including landraces take two to eleven months for the crop maturity (Kuleshov, 1933). Synchrony in flowering is the most important trait because of which particular landrace or breeding line could be easily maintained for breeding and particular hybrid combination or other line can become a cultivar in addition to the traits of high yield and tolerance to a variety of stresses. Asynchrony is the contrasting trait because of which huge diversity of the maize genetic resources arrives in nature, but the same trait of asynchrony in the flowering of the maize makes the line unsuccessful in self-perpetuation and causing consequent extinction of the lines and strains.

Active flowering duration too determined as the difference between 100% and 25% population of the maize hybrid although the active anthesis in a tassel lasts in about five to eight days which varies in temperature, soil moisture-nutrient level and hybrid to hybrid. Besides, peak anthesis happens on the third day of anthesis. Anthesis initiates first at about the central region of the principal rachis of the tassel; then, spreads bi-directionally. A little bit lagging after initiation of anthesis on tassel branches than the principal rachis. Very bottom anthers are very late to anthesis (Personal experience). Maize breeders seek hybrids with minimum ASI for its success which they herald at the time of the release of commercial cultivars.

Kernel number estimating equation could not have high r-square since large numbers of populations were grown in the same trial or next trials nearby (Table 3). Some hybrids with highest kernel numbers might have formed from the pollen of the next hybrids in the same trial or neighboring trial. Edmeades and Daynard (1979) first time reported that how many kernels will set in an ear of the maize plant is positively correlated to the rate of photosynthesis per plant during anthesis. The equation extracted from their experiment has been used to predict kernel number per plant and then incorporated into the original version of CERES-Maize (Jones and Kiniry, 1986). Andrade et al. (1999) too worked in this area. Struik et al (1986) reported that prevailing temperature, photoperiod and synchrony between anther dehiscence and silking are some of the determinants of kernel numbers in addition to plant photo-assimilate supply. Temperature and photoperiod like environmental and physiological factors influence the floral development and that eventually influences the kernel number determination in the maize ears (Herrero and Johnson, 1981; Hall et al., 1982; Struik et al., 1986; Bassetti and Westgate, 1993a). Several scientists have worked to find useful trait and equation for grain yield estimation through remote sensing (Guindin-Garcia, 2010) and pollen shed density (Westgate et al., 2003). But, for breeding and selection of superior maize, highly reliable diagnostic phenotypic naked eye marker could not come. So, efforts were applied to explore flowering or floral traits.

Rice has been studied extensively in Japan in response to natural start of cool temperature during flowering and grain filling. The studies reported that chilling damaged kernel set in rice. Similarly, autumn planted maize in subtropical region like Nepal and Gangatic plain in India faces such chilling stress during physiology from preparation of tassel emergence to pollination, fertilization to early grain filling. Female ovule fertility is fairly tolerant in a moderate cold stress; but, pollens are sensitive in rice (Hayase et al., 1969; Dupuis and Dumas, 1990). Sensitiveness of pollens and tolerance of ovule in chilling stress can be homologous in maize since maize is also tropical origin. The susceptibility of the pollens can subsequently reduce fusion of male gamete to egg cell for zygote formation maize.

Abbreviations used

PP: plant population; HPP: hybrid plant population; AV NKS: average number of kernels set;

Supplementary file:

Floral traits of single cross hybrids of yellow maize in winter in subtropical Nepalese Himalayan foot plain.

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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)**

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

Table 2: Grain yield (Y, in t ha^{-1}) estimating polynomial regression equations from flowering traits. The equations involve flowering traits as independent variables: The regression equations estimate grain yield of newly bred hybrids in t ha^{-1}. The regression equations have been formulated for plant population density of 57,143 per hectare in autumn planted spring harvested maize

$= -2450 + 97.7\ TSS - 1.294*TSS^2 + 0.005704*(TSS)^3$,	$r^2 = 36.1\%$	(Eq1)
$= -11922 + 507.3*TSS25 - 7.183*TSS25^2 + 0.03387*(TSS25^3$,	$r^2 = 65.8\%$	(Eq2)
$= -2856 + 115.3*TSS50 - 1.545*TSS50^2 + 0.006892*(TSS50)^3$,	$r^2 = 44.2\%$	(Eq3)
$= -2233 + 87.49*TSS75 - 1.136*TSS75^2 + 0.004914*(TSS75)^3$,	$r^2 = 46.7\%$	(Eq4)
$= -7748 + 295.2*TSS100 - 3.739*TSS100^2 + 0.01577*(TSS100)^3$,	$r^2 = 46.8\%$	(Eq5)
$= -3852 + 148.0*ANTH - 1.841*ANTH^2 + 0.007619*ANTH^3$,	$r^2 = 44\%$;	(Eq6)
$= -12088 + 487.8*ANTH25 - 6.552*ANTH25^2 + 0.02931*ANTH25^3$;	$r^2 = 56\%$	(Eq7)
$= -2922 + 110.5*ANTH50 - 1.387*ANTH50^2 + 0.00579*ANTH50^3$,	$r^2 = 45\%$	(Eq8)
$= -1948 + 69.1*ANTH75 - 0.809*ANTH75^2 + 0.003136*ANTH75^3$,	$r^2 = 50\%$	(Eq9)
$= -450.5 + 11.57*ANTH75 - 0.07258*ANTH75^2$,	$r^2 = 50\%$	(Eq10)
$= -2078 + 71.71*ANTH100 - 0.8180*ANTH100^2 + 0.003098*ANTH100^3$,	$r^2 = 58\%$	(Eq11)
$= -7216 + 254.6*SILK - 2.984*SILK^2 + 0.01164*SILK^3$,	$r^2 = 46\%$	(Eq12)
$= -7368 + 277.5(SILK25) - 3.475(SILK25)^2 + 0.01450(SILK25)^3$,	$r^2 = 32\%$	(Eq13)
$= -7574 + 273.6(SILK50) - 3.287(SILK50)^2 + 0.01315(SILK50)^3$,	$r^2 = 31\%$	(Eq14)
$= -10508 + 374.4(SILK75) - 4.440(SILK75)^2 + 0.01754(SILK75)^3$,	$r^2 = 31\%$	(Eq15)
$= -1497 + 48.28(SILK100) - 0.5139(SILK100)^2 + 0.001818(SILK100)^3$,	$r^2 = 27\%$	(Eq16)
$=40 - 3.39*(S\ SN) + 0.0579*(S\ SN)^2 - 0.000268*(S\ SN)^3$,	$r^2 = 26\%$	(Eq17)
$= -2131 + 63.97(S\ SN25) - 0.6349(S\ SN25)^2 + 0.002094(S\ SN25)^3$,	$r^2 = 33\%$	(Eq18)
$= -1044 + 29.43(S\ SN50) - 0.2729(S\ SN50)^2 + 0.000840(S\ SN50)^3$,	$r^2 = 26\%$	(Eq19
$= -3004 + 80.54(S\ SN75) - 0.7157(S\ SN75)^2 + 0.002115(S\ SN75)^3$,	$r^2 = 27\%$	(Eq20)
$= -1506 + 37.74(SSN100) - 0.3123(S\ SN100)^2 + 0.000859(S\ SN100)^3$,	$r^2 = 11\%$	(Eq21)

(Where S SN is days required for silk senescence of percent population).

Table 3: Average total kernels per plant (Yn) estimating equations: the equations have been developed in condition of plant population is 57,143 ha^{-1}. Yn is in fact an average number of functional egg cells and zygotes per plant that are successful to pull photo-assimilates for kernel development. The reproductive trait is independent variable (X).

The estimating equations have been formulated from ASI used in the equation is calculated from five sampled plants in each plot.

$Yn = - 159386 + 5456*SILK - 61.82*SILK^2 + 0.2325*SILK^3$, $r^2 = 36.8\%$; (Eq22)

$Yn = 494.6 - 82.67*ASI - 24.85*ASI^2 - 1.552*ASI^3$, $r^2 = 32\%$ (Eq23)

$Yn = - 18090 + 480\ ANTH5 - 3.24\ ANTH5^2 + 0.0019\ ANTH5^3$, $r^2 = 33.7\%$ (Eq24)

Table 4: Mean values and DMRT of tassel emergence and interval to anthesis of the maize hybrids grown in Nepalese subtropical winter.

Entry	Grain yield t ha^{-1}		TSS 5		TSS 25%	TSS 50%	TSS 75%	TSS 100%	TSS 100-25	TAI 25	TAI 50	TAI 75	TAI 100
8	12.54	A	79.1	A	75.3	78.3	80.7	83.3	8.0	3.3	2.0	1.3	1.7
12	11.80	A	72.5	B-D	69.3	73.3	75.3	78.7	9.3	3.3	1.7	2.0	3.3
11	11.55	A	74.9	A-C	72.3	74.3	76.3	78.3	6.0	1.3	1.7	2.3	3.7
13	11.31	AB	71.9	CD	68.0	70.0	73.0	74.0	6.0	6.3	6.3	5.3	7.3
5	11.05	AB	76.9	AB	74.0	76.7	80.0	83.0	9.0	4.3	4.0	3.0	3.7
6	11.02	AB	72.6	B-D	68.0	69.7	72.7	77.3	9.3	4.0	4.7	4.0	1.7
10	9.78	A-C	75.3	A-C	70.7	72.3	74.3	79.3	8.7	4.3	5.3	6.0	4.7
1	9.75	A-C	79.5	A	73.7	77.0	80.3	83.0	9.3	5.0	4.0	2.7	4.3
7	9.70	A-C	76.3	A-C	73.3	75.3	78.0	80.7	7.3	2.3	2.7	1.7	2.7
14	9.64	A-C	79.5	A	74.7	79.7	82.0	84.3	9.7	3.7	2.3	1.7	4.7
2	9.47	A-C	74.3	BC	67.3	71.7	75.0	80.7	13.3	5.3	3.7	6.7	3.3
9	9.30	A-C	74.3	BC	70.7	74.0	75.7	77.7	7.0	3.3	2.7	3.0	3.3
15	9.17	A-C	77.2	AB	73.7	77.0	79.7	82.7	9.0	3.3	3.3	4.3	7.7
4	7.87	BC	66.8	E	65.3	66.3	67.7	72.0	6.7	4.7	6.0	6.7	4.3
3	7.03	C	68.8	DE	65.3	66.7	68.3	72.3	7.0	3.3	3.7	4.0	3.0
Mean	10.07		74.7		70.8	73.5	75.9	79.2	8.4	3.9	3.6	3.6	4.0

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).

Table 5: Mean values and DMRT of anthesis of first earliest 25, second 25, third 25 and terminal 25% population of the maize hybrids grown in Nepalese subtropical winter.

Entry	ANTH 5		ANTH25		ANTH50		ANTH75		ANTH100		ANTH100-25	
8	83.1	ABC	78.7	A	80.3	AB	82.0	ABC	85.0	A-D	6.3	B
12	75.1	EFG	72.7	DEF	75.0	CDE	77.3	DE	82.0	CDE	9.3	AB
11	77.7	C-F	73.7	CDE	76.0	CD	78.7	CD	82.0	CDE	8.3	AB
13	76.9	D-G	74.3	CDE	76.3	CD	78.3	CD	81.3	C-F	7.0	B
5	81.1	A-D	78.3	AB	80.7	AB	83.0	AB	86.7	ABC	8.3	AB
6	75.7	D-G	72.0	EF	74.3	DE	76.7	DE	79.0	DEF	7.0	B
10	79.3	B-E	75.0	B-E	77.7	BCD	80.3	A-D	84.0	BCD	9.0	AB
1	84.3	AB	78.7	A	81.0	AB	83.0	AB	87.3	ABC	8.7	AB
7	79.5	B-E	75.7	A-D	78.0	BC	79.7	BCD	83.3	BCD	7.7	B
14	85.3	A	78.3	AB	82.0	A	83.7	A	89.0	AB	10.7	AB
2	79.4	B-E	72.7	DEF	75.3	CDE	81.7	ABC	84.0	BCD	11.3	AB
9	76.6	D-G	74.0	CDE	76.7	CD	78.7	CD	81.0	C-F	7.0	B
15	83.1	ABC	77.0	ABC	80.3	AB	84.0	A	90.3	A	13.3	A
4	72.1	G	70.0	FG	72.3	EF	74.3	EF	76.3	EF	6.3	B
3	72.4	FG	68.7	G	70.3	F	72.3	F	75.3	F	6.7	B
Mean	78.8		74.6		77.1		79.6		83.1		8.5	

Table 6: Mean values and DMRT of days for silking of the maize hybrids grown in Nepalese subtropical winter.

Entry	SILK5		SILK25		SILK50		SILK 75		SILK 100		SILK 100-25	
8	84.9	ABC	78.7	CD	82.3	A-D	85.0	AB	94.7	AB	16.0	ABC
12	78.7	CDE	76.3	DEF	78.3	DE	80.3	BC	83.7	CDE	7.3	D
11	82.2	B-E	76.3	DEF	79.0	CDE	81.3	BC	84.3	CDE	8.0	CD
13	82.3	B-E	76.3	DEF	78.3	DE	81.3	BC	86.7	B-E	10.3	BCD
5	85.9	AB	81.7	ABC	83.7	AB	88.7	A	95.0	AB	13.3	A-D
6	78.7	CDE	76.3	DEF	78.3	DE	80.7	BC	86.3	B-E	10.0	BCD
10	82.5	B-E	77.0	DE	79.3	CDE	81.7	BC	85.7	CDE	8.7	CD
1	91.0	A	82.7	A	85.0	A	88.3	A	101.3	A	18.7	A
7	84.3	A-D	77.3	DE	80.0	B-E	82.3	BC	87.7	B-E	10.3	BCD
14	86.6	AB	79.0	BCD	83.0	ABC	84.7	AB	90.0	BCD	11.0	A-D
2	78.9	CDE	74.0	EF	78.0	DE	82.0	BC	85.7	CDE	11.7	A-D
9	88.1	AB	82.0	AB	84.7	A	87.3	A	92.7	ABC	10.7	BCD
15	87.2	AB	78.0	D	81.3	A-D	84.0	AB	95.0	AB	17.0	AB
4	77.4	E	76.0	DEF	78.3	DE	80.3	BC	83.0	DE	7.0	D
3	77.7	DE	73.3	F	76.0	E	78.0	C	80.7	E	7.3	D
Mean	83.1		77.7		80.4		83.1		88.8		11.2	

Table 7: Mean values and DMRT of ASI and average total kernels (AV KNS) per plant each of the fifteen maize hybrids grown in Nepalese subtropical winter.

Entry	ASI 5	ASI 255	ASI 50	ASI 75	ASI 100	TOT KNLS
8	1.8 DEF	0.0 E	2.0 DE	3.0 B-E	9.7 ABC	721.2 A
12	3.5 B-E	3.7 BCD	3.3 B-E	3.0 B-E	1.7 DE	542.4 BCD
11	4.5 B-E	2.7 B-E	3.0 CDE	2.7 B-E	2.3 DE	524.9 BCD
13	5.5 BC	2.0 CDE	2.0 DE	3.0 B-E	5.3 B-E	488.8 B-E
5	4.9 BCD	3.3 B-E	3.0 CDE	5.7 ABC	8.3 A-D	599.9 AB
6	3.0 CDE	4.3 BCD	4.0 BCD	4.0 B-E	7.3 B-E	571.2 BC
10	3.2 CDE	2.0 CDE	1.7 DE	1.3 CDE	1.7 DE	517.3 BCD
1	6.7 B	4.0 BCD	4.0 BCD	5.3 A-D	14.0 A	431.5 CDE
7	4.9 BCD	1.7 CDE	2.0 DE	2.7 B-E	4.3 CDE	536.1 BCD
14	1.3 EF	0.7 DE	1.0 E	1.0 DE	1.0 E	454.1 CDE
2	-0.5 F	1.3 CDE	2.7 DE	0.3 E	1.7 DE	452.9 CDE
9	11.5 A	8.0 A	8.0 A	8.7 A	11.7 AB	513.7 BCD
15	4.1 B-E	1.0 CDE	1.0 E	0.0 E	4.7 CDE	440.1 CDE
4	5.3 BC	6.0 AB	6.0 AB	6.0 AB	6.7 B-E	348.9 E
3	5.3 BC	4.7 BC	5.7 ABC	5.7 ABC	5.3 B-E	404.8 DE
Mean	4.3	3.0	3.3	3.5	5.7	503.2

Table 8: Mean values and DMRT of days for silk senescence (S SEN) of the maize hybrids grown in winter in subtropical Nepalese Himalayan foot plain.

Entry	S SEN 5	S SEN25	S SEN50	S SEN75	S SEN100	ACT S SEN 100-25[A]
8	103.5 ABC	97.3 CDE	106.0 BCD	111.7 BCD	117.3 A-F	20.0
12	94.7 CD	90.0 FG	93.7 FG	101.3 E	109.7 EF	19.7
11	102.5 ABC	93.0 EFG	102.0 CDE	107.3 CDE	114.7 B-F	21.7
13	98.8 BCD	93.0 EFG	98.0 EF	104.0 DE	112.3 C-F	19.3
5	106.7 AB	99.7 BCD	110.3 AB	116.0 AB	125.3 AB	25.7
6	93.1 D	90.3 FG	95.0 EFG	102.3 E	108.7 F	18.3
10	98.6 BCD	94.3 DEF	100.7 DEF	105.3 DE	110.7 EF	16.3
1	110.1 A	106.0 A	115.0 A	119.7 A	126.7 A	20.7
7	100.1 BCD	92.7 EFG	100.3 DEF	106.3 CDE	111.7 C-F	19.0
14	110.5 A	102.7 ABC	108.3 ABC	113.7 ABC	123.3 ABC	20.7
2	96.2 CD	90.0 FG	96.0 EFG	102.0 E	106.3 F	16.3
9	109.7 A	104.7 AB	110.7 AB	115.3 AB	123.0 A-D	18.3
15	106.3 AB	102.0 ABC	108.7 ABC	113.7 ABC	120.7 A-E	18.7
4	94.5 CD	92.0 EFG	95.3 EFG	102.0 E	107.3 F	15.3
3	92.7 D	87.3 G	90.7 G	99.7 E	111.3 DEF	24.0
Mean	101.2	95.7	102.0	108.0	115.3	19.6

[A] It implies for active silk senescence duration that starts from silk initiation of first earliest 25% HPP to terminal 25% HPP.

Table 9: Mean values and DMRT of interval days between silk senescence (S SEN) and silk initiation (S INI) of the maize hybrids grown in Nepalese subtropical winter.

Entry	S SEN-S INI 25		S SEN-S INI 50		S SEN-S INI 75		S SEN-S INI 100		POP SEN 100%	
8	18.7	ABC	23.7	B-E	26.7	A-E	22.7	BCD	175.3	D
12	13.7	C	15.3	H	21.0	EF	26.0	A-D	181.7	A
11	16.7	C	23.0	B-F	26.0	A-F	30.3	ABC	179.0	ABC
13	16.7	C	19.7	E-H	22.7	C-F	25.7	A-D	180.0	AB
5	18.0	ABC	26.7	ABC	27.3	A-D	30.3	ABC	178.3	A-D
6	14.0	C	16.7	GH	21.7	DEF	22.3	CD	178.3	A-D
10	17.3	BC	21.3	C-G	23.7	B-F	25.0	BCD	180.0	AB
1	23.3	A	30.0	A	31.3	A	25.3	A-D	178.3	A-D
7	15.3	C	20.3	D-H	24.0	B-F	24.0	BCD	175.7	CD
14	23.7	A	25.3	A-D	29.0	AB	33.3	A	176.3	CD
2	16.0	C	18.0	FGH	20.0	F	20.7	D	176.7	BCD
9	22.7	AB	26.0	ABC	28.0	ABC	30.3	ABC	177.0	BCD
15	24.0	A	27.3	AB	29.7	AB	25.7	A-D	176.7	BCD
4	16.0	C	17.0	GH	21.7	DEF	24.3	BCD	175.3	D
3	14.0	C	14.7	H	21.7	DEF	30.7	AB	175.0	D
Mean	18.0		21.7		25.0		26.4		177.6	

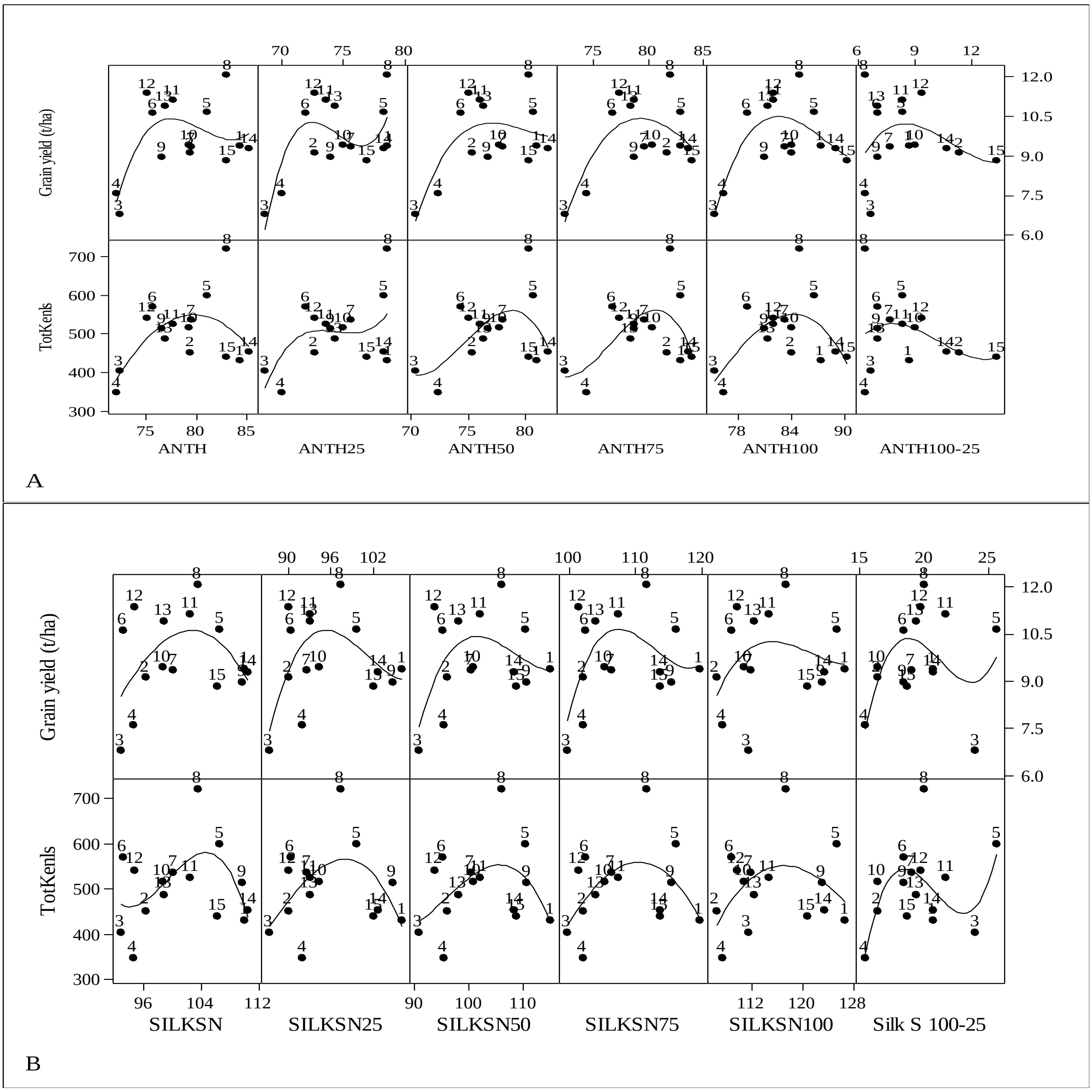

Figure 1: Correlation graphics of yield and kernel numbers with floral traits.