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Analysis of chlorophyll content and its correlation with yield attributing traits on early varieties of maize (*Zea mays* L.)

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ABSTRACT

Chlorophyll has direct roles on photosynthesis and hence closely relates to capacity for photosynthesis, development and yield of crops. With object to explore the roles of chlorophyll content and its relation with other yield attributing traits a field research was conducted using fourteen early genotypes of maize in RCBD design with three replications. Observations were made for Soil Plant Analysis Development (SPAD) reading, ear weight, number of kernel row/ear, number of kernel/row, five hundred kernel weight and grain yield/hectare and these traits were analyzed using Analysis of Variance (ANOVA) and correlation coefficient analysis. SPAD reading showed a non-significant variation among the genotypes while it revealed significant correlation with no. of kernel/row, grain yield/hectare and highly significant correlation with no. of kernel row/ear and ear weight which are the most yield determinative traits. For the trait grain yield/ha followed by number of kernel row/ear genotype ARUN-1EV has been found comparatively superior to ARUN-2 (standard check). Grain Yield/hectare was highly heritable (>0.6) while no. of kernel / row, SPAD reading, ear weight, number of kernel row/ear were moderately heritable (0.3-0.6). Correlation analysis and ANOVA revealed ARUN-1EV, comparatively superior to ARUN-2(standard check), had higher SPAD reading than mean SPAD reading with significant correlation with no. of kernel/row, no. of kernel row/ear, ear weight and grain yield/ha which are all yield determinative traits . This showed positive and significant effect of chlorophyll content in grain yield of the maize.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops of the world. It has remarkable production potential in Nepal and is the second staple food in Nepal after paddy. After paddy, maize is important cereal crop of Nepal after rice in terms of area, production and productivity (MoAD, 2013/14). At present, the Maize sown area in Nepal is 9,28,761 ha and total production is 22,83,222 metric tons with productivity of 2458 Kg/ha (MoAD, 2014). In Chitwan, maize covers total area of 9750 ha with the total production of 29,250 metric tons and productivity of 3,000 Kg/ ha (MoAD, 2014). Chitwan is a terai area and out of total maize cultivated area in Terai region, 95.95 % area is under

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improved and 4.05 % area under local maize. Maize exhibit sequential order of yield components development namely number of ear/plant, number of kernel/row, number of kernel row/ ear and hundred kernel weights (Viola *et al.*, 2004). There has been various study by different scholars about the yield attributing traits in maize. Among them chlorophyll content is one of the important attributing trait as it relates to photosynthesis capacity and stages of crop development. It is an important matter to study chlorophyll content and its significant roles in yield and production of maize in developing country like Nepal where there has not been much study in molecular level in case of maize and maize breeding. Chlorophyll accounted for more than 98% of gross primary production variation in maize (Gitelson *et al.*, 2008). The chlorophyll content of leaf has been suggested as the community property and has proportional relation to predict productivity. Not much information about the combining ability and genetic parameters about chlorophyll content of three-ear-leaves has been available yet. However, to evaluate the combining ability, reciprocal effect and genetic parameters for chlorophyll content of three-ear-leaves and how is it correlated with maize yield and other yield attributing traits was the objective of this research. By measuring the chlorophyll content, we can indirectly assess the potential taking up infrared radiation and the ability of a leaf to stay green (Araus *et al.*, 2008). The ratio of absorbance of radiation at 650 nm (chlorophyll absorbance peak) and at 940 nm (non-chlorophyll absorbance) can be calculated by Soil Plant Analysis Development (SPAD) meter SPAD-502. SPAD reading shows a positive relation with yield in maize as a result of transport of energy from photosynthesis due to increased production or shows negative relation with yield if energy is remobilized from chlorophyll. Any crop improvement is influenced by both genotypic variation and phenotypic variation. Degree of variability is an essential tool for effective selection. The phenotypic variation is affected by genotypic variation, environmental variation and interactions of both kinds (Lee & Lamkey, 2006). The total variance of a given character is its phenotypic variance (σ^2_p) and environmental variance ($\sigma^2 e$) attributed to environmental conditions. Determination of genotypic and phenotypic correlation is very fundamental step in the formulation and implementation of various breeding programs and activities. Correlation measures the degree of association, genetic or non-genetic, between two or more characters and is measured by a correlation coefficient (Hallauer & Miranda, 1988). In plant breeding, generally two types of correlations are discussed viz. genotypic correlation and phenotypic correlation. Phenotypic correlation (r_p) encompasses both genetic and environmental effects while the association of breeding values (i.e., additive genetic variance) of the two characters is genotypic correlation. Both of the correlations measure the extent how closely linked genes cause co-variation in two different characters (Hallauer & Miranda, 1988).The study was conducted to determine how chlorophyll content of three ear leaf plays role in maize yield and how it is correlated with other yield attributing traits. The research was conducted by taking fourteen genotypes including one standard check variety with following objectives:

- To study the chlorophyll content and its roles on maize productivity
- To study the correlation between chlorophyll content and other yield attributing traits of maize.

MATERIALS AND METHODS

Research site

Field experiment was conducted at research field of National Maize Research Program (NMRP) from 2 2 n d October 2014 to 12th April, 2015. The location is at 27°37′ N latitude and 84°25′E longitude with an elevation of 228 meters above sea level (NMRP, Rampur).

Climatic condition and season

The research site was climatically humid and sub-tropical. The average annual rainfall was 2000 mm (NMRP, Rampur). The meteorological data (Figure 1) were obtained from meteorological station, National Maize Research Program, (NMRP), Rampur.

Experimental details/materials/selection of genotype

Randomized Complete Block Design (RCBD) was laid with three replications and fourteen maize genotypes as treatments. These genotypes were allocated randomly to the fourteen plots of each replication. The plot size was 5.0 m × 3m with inter row spacing of 75cm and and intra row spacing of 25 cm, respectively. Each genotype was sown in each plot with four rows each of 5 m length. All genotypes were obtained from NMRP, Rampur. Following are the genotypes used in the research:

Entry no.	Genotype	Entry no.	Genotype
1.	ACROSS- 2401	8.	ARUN-2(standard check)
2.	RAJAHAR LOCAL	9.	FARMERS VARIETY
3.	S97TEYGHAYB(3)	10.	ZM-621/POOL-15
4.	POP-445/POP-446	11.	EEYC1
5.	ARUN-1EV	12.	KY/Pool-17
6.	R.C/POOL-17	13.	Pool-27
7.	SO3TETEY/LN	14.	Pool-15

Crop management

Land preparation was performed by ploughing two times followed by land leveling. Farm yard manure (FYM) was applied at the rate of 10 tons/ha and chemical fertilizers as per general recommendation for maize were applied at the rate of 120:60:40 NPK kg/ha of Nitrogen, Phosphorus and potassium respectively. Full dose of Phosphorus, Potassium and half dose of Nitrogen were applied at the time of sowing as basal application. Sowing was done on 22nd October, 2014 by manually operated sowing machine. Other half dose of Nitrogen was applied in splits of two doses doses during first and second weeding. Two hand weedings were carried out at 20-25 DAS. Thinning was done during first 15- 20 DAS. Earthing up was done as second weeding. The maize borer was controlled by placing granules of Phorate in top folding leaf. Phorate is an organophosphate used as an insecticide and acaricide.

Data collection

All the observation was recorded from five randomly selected plants of each plot and average values were taken for analysis. Observations were taken for major traits viz. SPAD measures, number of kernels per row, number of kernel rows per ear, ear weight (gm), 500 kernel weight (gm) and grain yield per hectare with moisture adjustment at 14%.

Chlorophyll Nitrogen implying SPAD measures (SPAD reading)

Chlorophyll content of leaf was measured by using Soil Plant Analysis Development (SPAD) meter (SPAD 502, Minolta, Japan). Five plants were randomly selected from each plot to measure chlorophyll content.

Number of kernels/row

Number of kernels/ row was counted and recorded for five randomly selected ear and average value was taken.

Number of kernel row/ear

Number of kernel rows/ ear was counted and recorded for five randomly selected ear and average value was taken.

Ear Weight (gm)

Ear weight was measured after harvesting the cob and its weight was measured for individual cob.

Five-Hundred kernel weights (g)

Five Hundred kernel weights of samples was recorded along with their moisture content by using moisture meter and then it was converted to fourteen percent (14%) moisture content by using formula:

$$\text{500-Kernel weight} = \frac{100 - \text{Moisture content \%}}{\text{100-Required moisture\% (14\%)}} \times \text{(500-kernel weight)}$$

Grain yield

To calculate grain yield, yield/plot was converted into grain yield/ ha. Grain Yield/ plot was converted to 14% moisture by using formula as above and then it was converted to grain yield/ha at 14% moisture by using given formula:

$$GY = \frac{\text{Yield / plot at 14\% moisture (kg)}}{\text{Plot size in } m^2} \times 10000\ m^2$$

Phenotypic and genotypic variance

Formula given by (Lush, 1940) and (Chaudhary & Prasad, 1968) was used to calculate phenotypic and genotypic variance.

Genotypic variance = $\sigma^2 g$ = (TMSS – EMSS) / R

Error variance = $\sigma^2 e$

Phenotypic variance = $\sigma^2 p = \sigma^2 g + \sigma^2 e$

Where,

TMSS is treatment mean sum of square
EMSS is error mean sum of square
R is number of replication

Genotypic and phenotypic coefficient of variation (GCV and PCV)

They are expressed as percentage. According to (Burton & Devane, 2008):
Genotypic coefficient of variation (GCV) = $(\sigma g/\bar{X}) \times 100$
Phenotypic coefficient of variation (PCV) = $(\sigma p/\bar{X}) \times 100$
Where,
σg = Genotypic standard deviation
σp = Phenotypic standard deviation
$\bar{X}$ = General mean of the trait

As indicated by (Sivasubramanjan & Menon, 1973), GCV and PCV are categorized as follows:

0 – 10 % : Low
10 – 20 % : Moderate
>20 % : High

Broad sense heritability (h_{bs}^2)

Hanson *et al* . , (1956) estimated broad sense heritability as the ratio of genotypic variance (V_g) to the phenotypic variance (V_p) and expressed in percentage.

Broad sense heritability (h_{bs}^2) = (V_g/V_p)

Robinson *et al.*, (1949) categorized broad sense heritability as follows:

0 – 0.30 : Low
0.30 – 0.60 : Moderate
> 0.60 : High

Genetic advance (GA)

It was calculated by using the following formula given by (Robinson *et al.*, 1949).

$$GA = i.\ \sigma p.h_{bs}^2$$

Where,

i = Efficacy of selection (2.06 at 5% selection intensity)
σp = Phenotypic standard deviation
h_{bs}^2 = Broad Sense Heritability

Genetic advance as percent of means (GAM)

GA as per cent of mean (GAM) = $(GA/\bar{X}) \times 100$
GA = Genetic advance
$\bar{X}$ =General mean of the trait

Johnson *et al.*, (1955) categorized GAM as follows:

0 - 10 % : Low
10 -20 % : Moderate
> 20 % : High

Statistical analysis

Analysis of variance was carried out by using statistical software GENSTAT, MINITAB version 20 as well as correlation analysis was done using SPSS version 21.

RESULTS AND DISCUSSION

Mean performance and analysis of variance

Mean values and significant levels of yield and yield attributing traits of fourteen maize germplasm are presented in Table 1. Significant results were observed for the for the traits grain yield/ha, ear weight, number of kernel/row, number of row kernel / ear, while non significant result was observed for SPAD reading and five hundred kernel weight.

Chlorophyll nitrogen implying SPAD measures (SPAD reading)

A non-significant difference ($P \leq 0.05$) in SPAD was found for the genotypes (Table 1). Maximum SPAD (51.74) had been showed by ARUN-2 (standard check) and minimum SPAD (44.59) had been showed by Pool-27.

Number of kernel rows/ear (NKRE)

The result showed significant difference ($P \leq 0.05$) in NKRE for the genotypes (Table 1). Maximum NKRPE (13.07) has been shown by ZM-621/POOL-15 and minimum (9.60) has been shown by KY/Pool-17.

Number of kernel/row (NKPR)

The result showed significant difference ($P \leq 0.05$) in NKPR for the genotypes (Table 1). Maximum NKPR (23.67) has been showed by FARMERS VARIETY and minimum (15.07) has been showed by KY/Pool-17. Variety S97TEYGHAYB(3), FARMERS VARIETY, ZM-621/POOL-15 , RAJAHAR LOCAL, POP-445/POP-446, ARUN-1EV, R.C/POOL-17, SO3TETEY/LN , EEYC1, Pool-27, Pool-15 were statistically similar to ARUN-2 (standard check) (Table 1).

Five Hundred kernel weights (FHKW)

The result showed that there was non-significant difference ($P \leq 0.05$) in FHKW for the genotypes (Table 1). Maximum FHKW (150.7) has been showed by ACROSS-2401 and minimum (112.0) has been showed by RAJAHAR LOCAL.

Grain yield Kg/Hectare (GYPH)

The result showed that there was significant difference ($P \leq 0.05$) in the grain yield kg/ha for the genotypes (Table 1). Among the tested genotypes ARUN-1EV has been found high yielder with grain yield of 2376 kg/ha and genotype KY/pool-17 with 164 kg/ha was low yielder genotype (Figure 2). The genotypes ARUN-1EV, S97TEYGHAYB (3), Farmers variety , SO3TETEY/LN, Pool-15, POP-445/POP-446 were found statistically at par with ARUN-2 (standard check) for grain yield kg/ha. The grain yield/ha in relation to genotypes has been shown in figure 2.

Broad sense heritability (hbs^2)

The considerable differences in heritability value for different characters were observed (Table 2). Among quantitative characters, high heritability (>0.60) has been observed for grain yield/ha

(0.67), Moderate heritability (0.30 to 0.60) has been found for number of kernel/row (0.344), SPAD reading (0.118), ear weight (0.45), number of kernel row/ear (0.376) while low heritability (<0.30) has been observed in five hundred kernel weight. (Viola *et al.*, 2004) reported high heritability for grain yield/ha which is very consistent with our finding. Grain yield/ha and ear height had better genotypic variability; better broad sense heritability along with better GA is considered the good estimates for effective selection of a trait. This depicted that visual selection based on these traits among the genotypes would be used for improvement of grain yield.

Genetic advance as percentage of mean (GAM)

GAM at five percent selection intensity exhibited greater differences for quantitative characters as represented in Table 2. High GAM having value more than 20% were estimated for ear weight and grain yield/ha. GAM between 10 and 20 % has been observed for number of kernel / row. Lower values were estimated for five hundred kernel weights, number of kernel row/ear, SPAD reading and ear length. GAM showed that ear weight, plant height and grain yield/ha were under control of additive genes. Alvi *et al.*, (2003) reported similar findings.

Phenotypic Coefficient of Variation and genotypic coefficient of variation (PCV and GCV)

The considerable difference in PCV and GCV values for different traits has been observed (Table 2). Among the studied quantitative traits, PCV and GCV values were estimated higher for grain yield/ha, ear weight had high PCV and moderate GCV, whereas five hundred kernel weights, number of kernel/row had moderate PCV values And number of kernel row/ear, SPAD reading had lower PCV and GCV value. The difference between GCV and PCV ranged from 3.747 to 10.913 and was lower for all traits studied. This showed the higher genetic effects in these parameters for variation. Higher broad sense heritability of all traits revealed that larger portion of variations is heritable to offspring.

Correlation coefficient

Analysis of correlation coefficient of yield related traits revealed some fundamental basis. Regression equation showed the relationship between the grain yield/ha with variables i.e. SPAD reading, number of kernel row/ear, number of kernel/row, ear weight and five hundred kernel weights. All traits showed positive correlation with grain yield/ha (Figure 3). This means that grain yield per hectare increases with increase in value of SPAD reading, number of kernel rows/ear, number of kernel/row, ear weight and five hundred kernel weight. Ibitome (2010) and Rafique et al. (2004) reported similar findings. Chlorophyll content by SPAD reading was found significantly correlated with number of kernel/row and number of kernel row/ear, ear weight were highly significant with SPAD reading. Thus, these traits have highly significant correlation with grain yield / hectare. The high direct effect of SPAD reading, number of kernel row/ear, number of kernel/row and ear weight appeared to be the main factor for their strong association with grain yield/plant. Hence direct selection for these traits would be effective. The traits grain yield/ha, number of kernel/row have shown higher heritability and also expressed highly positive and significant correlation coefficient. Thus, these traits are to be considered in selecting genotypes for better crop improvement and breeding programs.

CONCLUSION

Chlorophyll content and its correlation with yield attributing traits among the different maize genotypes was observed from this study which is very useful for their improvement of agronomically important traits. Grain yield is the very complex parameter in Maize. Any minor change in any component leads to the yield loss. Grain yield and related traits are very sensitive to any crops. Association of different yield and yield attributing traits are very important to know their direct and indirect effects on grain yield. In this study we emphasized to determine the correlation coefficient of the traits with chlorophyll content in order to understand and identify how chlorophyll play a vital role in selection and breeding for simultaneous improvement of genetic materials. For the trait grain yield ha^{-1} followed by number of kernel row/ear, ARUN-1EV has been found comparatively superior to ARUN-2(standard check). For yield attributing traits like number of kernel row/ear, number of kernel/row and five hundred kernel weight genotype SO3TETEY/LN, FARMERS VARIETY and ACROSS-2401 showed highest value respectively. Grain Yeild Per hectare was highly heritable .Similarly, SPAD reading, no. of kernel per row, number of kernel row per ear, ear weight were moderately heritable. Higher GCV and high GAM indicate efficient indirect selection for higher grain yield/ha based on these traits. Thus high GAM and GCV was observed in ear weight ,grain yield per hectare and ear height. Correlation analysis revealed that the traits number of kernel row per ear , no. of kernel per row and ear weight were the most yield determinative traits and hence, selection of these traits for further breeding program might bring an improvement in grain yield. SPAD reading for chlorophyll content showed positive significant correlation with grain yield. Correlation analysis and ANNOVA revealed ARUN-1EV, comparatively superior to ARUN-2 (standard check), had higher SPAD reading than mean SPAD reading with significant correlation with no. of kernel/row, no. of kernel row/ear, ear weight and grain yield/ha which are all yield determinative traits . This showed positive and significant effect of chlorophyll content in grain yield of the maize. Besides the correlation *inter se* associations also provide huge support on these six traits from all other yield related components. The variability shown by different genotypes for different yield attributing quantitative traits can be used for the development of the high yielding and better performing variety. From the research, selection of ARUN-1EV genotype was found to be reliable for further research and breeding program in early varieties of maize. SPAD reading exhibiting chlorophyll content was found significantly correlated with grain yield and yield attributing traits.

Table 1.Means different parameters of fourteen maize genotypes in Chitwan, Nepal, 2014/15

	Mean					
Genotype	SPAD	NKRPE	NKPR	EW(gm)	FHKW(gm)	GYPH(kg)
ACROSS- 2401	47.81	11.60[a]	17.53[bc]	64.7[bcd]	150.7	493[ef]
RAJAHAR LOCAL	47.17	12.40[a]	19.80[ab]	61.4[cd]	112.0	728[def]
S97TEYGHAYB(3)	45.37	12.67[a]	23.13[a]	81.1[abc]	120.7	2222[a]
POP-445/POP-446	48.01	12.67a	20.73[ab]	76.1[abc]	122.0	1922[abc]
ARUN-1EV	48.53	12.13[a]	21.67[ab]	86.0[ab]	134.0	2376[a]
R.C/POOL-17	45.27	11.60[a]	19.33[abc]	61.1[cd]	116.7	711[def]
SO3TETEY/LN	50.00	12.53[a]	21.13[ab]	87.2[ab]	138.7	2175[ab]
ARUN-2(std check)	51.74	11.60[a]	23.33[a]	93.9[a]	140.7	2232[a]
FARMERS VARIETY	49.59	11.33[a]	23.67[a]	88.7[ab]	132.7	1982[ab]
ZM-621/POOL-15	48.96	13.07[a]	20.53[ab]	73.2[abc]	117.3	820[def]
EEYC1	46.96	12.40[a]	21.93[ab]	81.3[abc]	126.0	1073[cdef]
KY/Pool-17	46.01	9.60[b]	15.07[c]	49.2[d]	119.0	164[f]
Pool-27	44.59	11.33[a]	20.93[ab]	64.7[bcd]	123.3	1278[bcde]

Pool-15	51.58	12.67[a]	20.67[ab]	88.3[ab]	130.0	1598[abcd]
Grand mean	47.97	11.97	20.68	75.5	127.4	1412
SEM (±)	1.907	0.529	1.434	7.20	9.97	281.5
$LSD_{0.05}$	ns	1.537*	4.169*	20.92**	ns	818.4**
CV %	6.9	7.7	12.0	16.5	13.5	34.5

*(significant), **(highly significant) & ns(non significant) at p=0.05. Treatment means bearing same letter are not significant different at p=0.05 by DMRT. SEM=Standard Error of Mean, LSD=Least Significant Difference & CV=Coefficient of Variance, SPAD=Spad Reading, NKRPE=Number of Kernel Row / Ear, NKPR=Number of Kernel / Row, EW=Ear Weight, FHKW=Five Hundred Kernel Weight, GYPP=Grain Yield / Plant, GYPH=Grain Yield / Hectare.

Table 2: Estimation of genetic parameters (GAM, PCV and GCV) for fourteen maize genotypes in Chitwan, Nepal, 2014/15

Variable	GCV	PCV	h_{bs}^2	GAM
SPAD	2.519	7.332	0.118	1.783
NKRPE	5.941	9.688	0.376	7.506
NKPR	8.7	14.831	0.344	10.513
EW	14.922	22.255	0.45	20.612
FHKW	3.43	13.977	0.06	1.734
GYPH	49.193	60.106	0.67	82.939

Vg=genotypic variance, Ve=environmental variance, Vp=phenotypic variance, GCV=genotypic coefficient of variation, PCV=phenotypic coefficient of variation, GAM=genetic advance as percentage of mean, h_{bs}^2=broad sense heritability, SPAD=Spad reading, NKRPE=number of kernel row/ear, NKPR=number of kernel/row, EW= Ear Weight, FHKW= Five hundred kernel weights, GYPH= grain yield/ha

Table3. Pearson's correlation coefficient between yield and yield attributing traits of fourteen genotypes of maize in Chitwan, Nepal, 2014/15

	SPAD	NKRE	NKPR	EW	FKW	GYPH
SPAD	1.00					
NKRE	0.45**	1.00				
NKPR	0.31*	0.53**	1.00			
EW	0.51**	0.58**	0.74**	1.00		
FKW	0.04ns	-0.11ns	-0.07ns	0.40**	1.00	
GYPH	0.31*	0.39**	0.59**	0.78**	0.35*	1.00

PAD= Spad reading, NKRPE=number of kernel row/ear, NKPR=number of kernel/row, EW= Ear Weight, FHKW= Five hundred kernel weights, GYPH= grain yield/ha

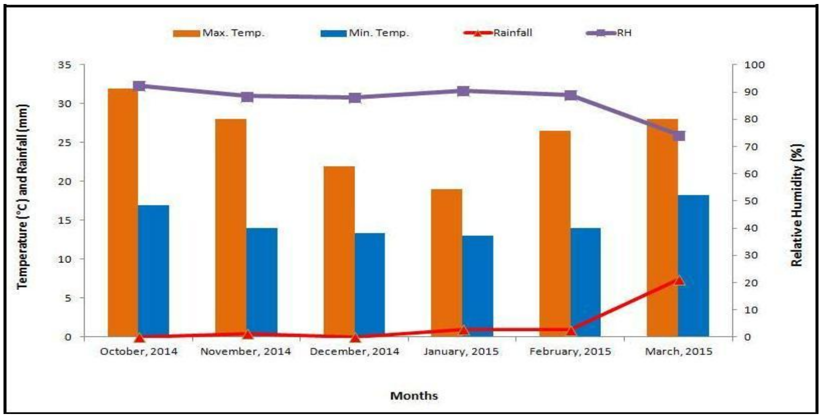

Figure1: Meteorological data during the growing period of Maize at Rampur Chitwan 2014/15

Chart of GYPH vs GENOTYPES

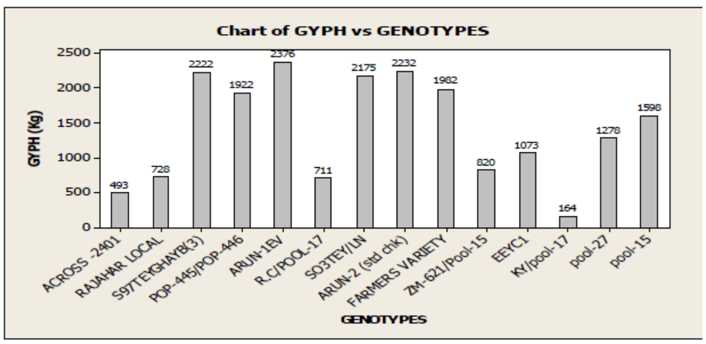

Figure 2: Genotypes with their yields (kg/ha)

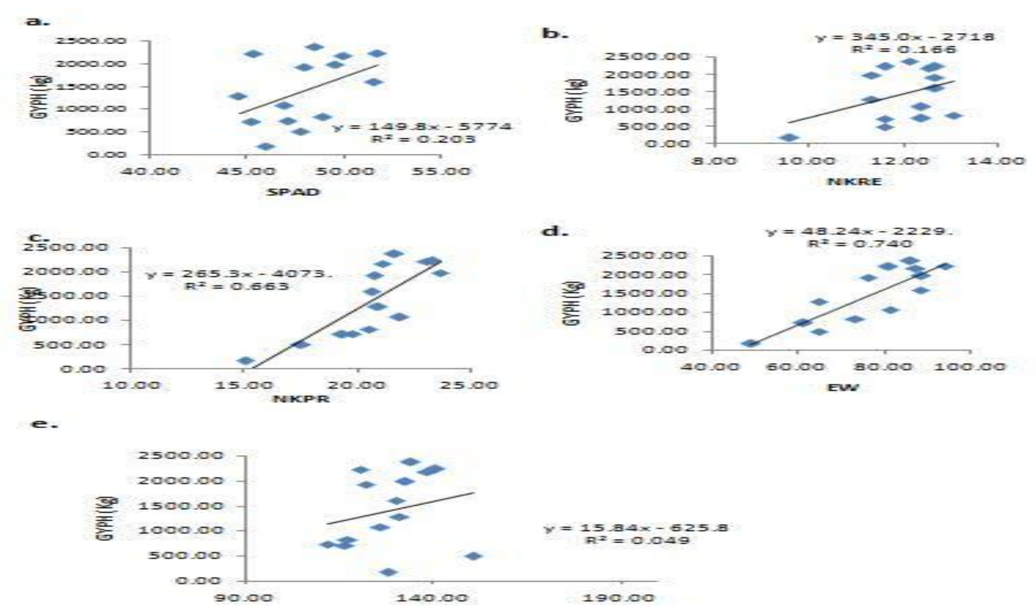

Figure 3. Estimated liner correlation (a) between SPAD reading and grain yield/ha (GYPH), (b) between NKRE (number of kernel row/ear) and grain yield/ha (GYPH), (c) between NKPR (number of kernel/row) and grain yield/ha (GYPH), (d) between EW (ear weight) and grain yield/ha (GYPH), (e) between FHKW (five hundred kernel weight) and grain yield/ha (GYPH)

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