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Chlorophyll Content in Leaves of Wheat as Influenced by Inorganic, Organic and Integrated Sources of Nutrient Application

Megha Vishwakarma[1], P. S. Kulhare[1] and G. S. Tagore[1*]

[1]Department of Soil Science and Agricultural Chemistry, College of Agriculture, JNKVV, Jabalpur (M.P.), India.

Authors' contributions

This work was carried out in collaboration between all authors. Author PSK designed the study, Author MV performed analyses the statistical analysis and search the literature. Author GST wrote the protocol of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Field experiments were conducted during winter season of 2018-19 and 2019-20 with three sources of nutrient *viz.,* inorganic, organics (FYM, VC and biofertilizers) and their integration as main treatments and five levels [S1-0 (0-0-0 kg NPK ha^{-1}), S2-100% (120-60-40 kg NPK ha^{-1}), S3-150% (180-90-60 kg NPK ha^{-1}), S4-200% (240-120-80 kg NPK ha^{-1}) and S5-Soil Test based (STV) NPK i.e. 149-176-33 kg ha^{-1} in split plot design with three replications. The chlorophyll content ('a', 'b' and total) in leaves and Soil Plant Analyzer Development (SPAD) value were recorded at crown root initiation (CRI), tillering, jointing and milking stage of wheat. The pooled data of findings revealed that the treatment with inorganic sources showed significant increase in the SPAD readings (9.62, 15.54, 23.77 and 29.83), chlorophyll 'a' (0.76, 0.83, 1.47 and 0.63 mg g^{-1} leaf tissue), 'b' (0.44, 0.78, 0.87 and 0.57 mg g^{-1} leaf tissue) and total (1.19, 1.64, 2.25 and 1.14 mg g^{-1} leaf tissue) chlorophyll content in leaves over organic source at all the growth stages.

All the levels of nutrient were significantly increased the chlorophyll content and SPAD value over control at all the stages except chlorophyll 'a' at jointing and milking stage. However, amongst the levels 150% and 200% NPK were found significantly superior to 100% NPK for SPAD value (8.32

**Corresponding author: E-mail: gstagore@gmail.com;*

and 8.71 at CRI and 12.56 and 12.19 at tillering), chlorophyll 'a' (0.73 and 0.70 mg g^{-1} leaf tissue at CRI), chlorophyll 'b' (0.46 and 0.45 mg g^{-1} leaf tissue at CRI, 0.68 and 0.71 mg g^{-1} leaf tissue at tillering and 0.53 and 0.59 mg g^{-1} leaf tissue at milking), respectively.
The interaction results suggested that the 200% NPK with inorganic and integrated sources significantly superior to 100% NPK for chlorophyll 'a' content at jointing and milking stage. The application of 150% and 200% NPK with inorganic source were found significantly higher over the same level of NPK with integrated source of nutrient for total chlorophyll content and SPAD value at all the growth stages except 150% NPK for total chlorophyll at jointing and milking stage and SPAD value at milking stage. The correlation between SPAD value and chlorophyll 'a', 'b', total were found significantly and positively at all growth stages. Coefficient of determination values between SPAD and chlorophyll content showed linear relationship at all the growth stages.

Keywords: SPAD; Chlorophyll; Interaction; Organic; Inorganic; INM.

1. INTRODUCTION

Wheat *(Triticum aestivum L.)* is the most important cereal in India and it is grown in an area of 30.42 m ha with annual production of 92.29 m t and productivity of 3034 kg ha^{-1}. While in Madhya Pradesh it is grown in an area of 5.91 m ha with the production of 17.69 m t and productivity of 2993 kg ha^{-1} [1]. Its productivity in country as well as the state is lower than other country such as China and Mexico (5 t ha^{-1}). The hybrid varieties producing around 5 t ha^{-1} of grain can remove about 110 kg N, 15 kg P, 129 kg K, 5 kg S, 2 kg Fe, 2 kg Mn, 200 g Zn and 150 g B ha^{-1} from the soil but the farmers are using N fertilizers mainly urea with inadequate P and K nutrients. This insufficient/imbalance fertilization deteriorated the soil fertility and reduced the productivity [2] as well as existed recommendation of N, P and K fertilizers failed to achieve optimum yield. The deficiency, particularly of N and of P is so acute in most of soils hence considered as pivot of agriculture production. To achieve that productivity, the management of soil health is necessary with the use of optimum N, P and K.

N is an essential constituent of chlorophyll and controls many aspects of plant metabolism and development and biochemical processes (Krouk et al., 2010). P plays a vital role photosynthesis, respiration, energy storage, and cell division/enlargement (Bakhsh *et al.,* 2008). K is closely associated with process of photosynthesis and transport of photosynthate to storage organs. A good supply of nitrogen to the plant stimulates root growth and development as well as uptake of other nutrient (Braddy and Weil, 2002).

Chlorophyll is one of the important pigment content which is used as an index of plant production capacity [3]. Chlorophyll pigments consist of two main types, 'a' and 'b'. Their contents relate closely to primary production because they absorb sunlight and convert sunlight, water, and carbon dioxide into carbohydrates and oxygen [4]. Chlorophyll 'a' is the principal pigment involved in the photosynthesis whereas chlorophyll 'b' is the accessory pigment, collecting the energy in order to pass into chlorophyll 'a'. Indication of high levels of chlorophyll content is a result of effective photosynthetic and metabolic activity. The amount of chlorophyll present in leaves depends to a large extent on the status of plant nutrition. Deficiencies of various mineral elements are known to disturb the development of chloroplast pigments in general and chlorophyll in particular in addition to reducing growth and yield. The readily availability of nutrient from fertilizers attributed to vigorous foliage growth, increased meristematic and more intense physiological activities in the plants which favoured the synthesis of more photo-assimilates [5] resulted maximum chlorophyll content of wheat. Thus, the value of leaf chlorophyll content can help to understand nutritional status of the plant, and scientifically guide the fertilizer management to ensure a good crop quality and yield. Whereas the relationship between the chlorophyll content of leaves and the actual photosynthetic canopy area is well documented, comparatively little information is available about the vertical distribution of important plant parameters including chlorophyll, a key crop biophysical characteristic [6], and nitrogen, crucial resource for plant development [7-8].

Farmyard manure (FYM), crop residues and bio fertilizers are advocated to improve N use efficiency, soil organic carbon, crop productivity and soil health (Liu et al., 2005). These

compounds can increase agricultural sustainability in terms of significant enhancement of vegetative growth, yield and nutrient uptake by improving the physicochemical properties of the soil and increase of beneficial microbial populations for plants and soil fertility [9-10]. Moreover, solubilisation of P and K, uptake of N and multiplication of extraradical hyphae biomass are affects promoted by biofertilizers that minimize negative impacts such as erosion and soil degradation [11]. The major limitation of organic and biological sources have low nutrient content, often has to deal with a scarcity of readily available nutrients in contrast to inorganic farming which relies widely available on soluble fertilisers and usually required in large quantity but not abundantly available. Thus, NPK requirements and management of these essential nutrients for crop production have become a focus of research into the interactions between them in terms of factors such as sources and levels. The interactive advantages of combining different sources of nutrient in one of the possible options to reduce the use of inorganic fertilizers, cost of cultivation and achieve sustained crop production and maintain better soil health [12]. In India, it is said that nutrient supplying capacity of soil declines steadily under continuous and intensive cropping system. Hence, judicious use of nutrient requires its synchronized application with crop requirement.

The traditional methods used to determine the amount of chlorophyll in the leaf require sampling and destruction of plant tissue, and the chlorophyll extraction and quantification processes are time-demanding. Soil plant Analyzer Development (SPAD) meter is used worldwide to manage crop nitrogen status and monitor crop activity which provides relative leaf chlorophyll or nitrogen content. The SPAD meter values (0-50) are proportional to amount of chlorophyll in the leaves sample [13]. The N concentration in plant leaves has strong and positive relationship with the SPAD values, being more evident in the later growth stages [14]. The leaf chlorophyll content shows also high correlation with SPAD results [15-16]. Such meters have been used to estimate foliar chlorophyll and N content in various species, such as rice [17], wheat [18], beans [19], and fruit trees [20]. The objective of the present investigation was to chlorophyll content of wheat at different growth stages and its relationship with SPAD.

2. MATERIALS AND METHODS

2.1 Experiment Details

A field experiment was carried out in the research field of Department of Soil Science and Agricultural Chemistry Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur during the 2018-2019. The soil of the experimental site was Typic Haplustert, clay in texture neutral in reaction, non-calcareous, medium in organic carbon content, medium in available nitrogen, phosphorus, and potassium and low in DTPA extractable Zn. The treatment comprised of 3 sources of nutrient M_1- inorganic, M_2- organics (FYM, VC and biofertilizers) and M_3- integrated (inorganic and organic) as main treatments and 5 fertility levels S_1- 0 (0-0-0 kg NPK ha^{-1}) S2-100% (120-60-40 kg NPK ha^{-1}), S3-150% (180-90-60 kg NPK ha^{-1}), S4-200% (240-120-80 kg NPK ha^{-1}) and S5-Soil Test based (STV) NPK i.e. 149-176-33 kg ha^{-1} as sub treatments were replicated thrice in a split plot design. The wheat crop (GW-366) sown on with spacing of 22.5 cm row to row. The observations were recorded from each plot at crown root initiation (CRI), tillering, flowering and milking stages of wheat.

2.1.1 Experimental materials

The inorganic source of nutrients were applied through Diammonium phosphate, urea, single super phosphate and muriate of potash. The nutrients with organic sources were applied through farm yard manure, vermicompost (VC), *Azotobacter* and phosphorous solubilizing bacteria (PSB). STV based nutrients with organic source was applied through wheat straw, *Azotobacter* and PSB. Well decomposed FYM, VC and wheat straw were applied by mixing with the soil well before 30 days of sowing. One third N of treatment and full dose of P and K were applied through fertilizer as basal application. The 1/3 N was applied at 21 days after sowing (DAS) and remaining 1/3 N was applied at 65 DASas per treatment during both the year. The total N by Kjeldahl digestion [21], P by spectrophotometer [22] and K by flame photometer [23] were determined and the result is depicted in Table 1.

2.1.2 Chlorophyll content measurement

The material was processed in the fresh state immediately after collection. After fine chopping, portions weighing 0.5 g were measured off on an analytical balance. The measured-off material

was then homogenized in a homogenizer with the addition of 10 ml of 80% acetone. A primary acetone extract containing all chloroplast pigments was obtained in this way. The extract was then centrifuged at 2500 rpm for 5 min. Since the concentration of pigments was in most cases too great for reading to be performed on a spectrophotometer, the obtained extract was diluted by adding 9 ml of 80% acetone per ml of extract. The extract produced in this way was subjected to reading on a spectrophotometer at 645 and 663 nm using acetone (80%) blank. The amount of chlorophyll 'a' and 'b' are determined using the formula given by Arnon [24].

Chl 'a'= ((12.7 A663)-(2.69 A645))
Chl 'b'= ((22.9 A645)-(4.68 A643))
Total chlorophyll (a+b) **=** ((20.2 (A645) +8.02(A 663))

Where, A = Absorbance

2.1.3 Estimation of chlorophyll using SPAD-502

The SPAD (Soil Plant Analysis Development) is a simple, rapid, and non-destructive method for evaluation of chlorophyll contents in leaves and can be used in the field and laboratory. The SPAD-502 measures the content of chlorophyll (CC) in the leaf, which is related to leaf greenness, by transmitting light from light emitting diodes (LED) through a leaf at wavelengths of 650 and 940 nm. Chlorophyll content measurements were carried out four times between flowering and the end of senescence on three flag leaves of wheat between 10 AM to 4 PM wholly expanded leaf from apex is chosen and clamped after avoiding the mid-rib portion into the sensor hold of the SPAD meter.

2.1.4 Statistical analysis

Data were analysed using SPSS for analysis of variance and Fisher's LSD multiple range test was employed for the means comparisons.

3. RESULTS AND DISCUSSION

3.1 SPAD Value at Critical Stages Using SPAD Meter

It can be seen from the data Table 2 that the SPAD value significantly increased with the application of inorganic (M_1) and integrated source (M_2) over organic source (M_3) at all stages. However, the inorganic source was also found significant over integrated source at jointing stage and at par at CRI, tillering and milking stages. Maximum SPAD value of 9.62, 15.54, 23.77 and 29.83 with the addition M_1 followed by 6.85, 9.21, 20.27 and 23.44 in M_3 and lowest 4.34, 5.85, 15.85 and 16.93 in M_2 at CRI, tillering, jointing and milking stages, respectively.

The application of S_3 (150% NPK) and S_4 (200% NPK) significantly increased the SPAD value over S_2 (100% NPK) at CRI and tillering stage. S_3 (150% NPK) and S_4 (200% NPK) were also found significantly superior to S_5 (STV based NPK) at CRI and milking stage. However, the S_2 and S_5 were found non-significant with each other at all stages. The treatment S_4 gave the highest value of SPAD (8.71, 12.19, 22.44 and 26.46) which was found at par with S_3 with the value of 8.32, 12.56, 22.04 and 26.46 at each stage, respectively.

The interaction effect were found to be significant for SPAD value for all the stages of wheat. The combination of M_1S_4 (200% NPK with inorganic source) of 12.33, 19.25, 27.97 and 37.93 was recorded the highest SPAD value, which was significantly superior to all the combination and at par with M_1S_5 (STV based NPK with inorganic source) of 11.63, 18.90, 25.46 and 31.80), M_1S_3 (150% NPK with inorganic source) 11.60, 18.85, 27.52 and 32.22 all were found at par among themselves. However, the lowest value found under M_2S_5 (STV based NPK with organic source) of 3.02, 5.69, 13.63 and 16.46 at each stage, respectively.

Table 1. Nutrient content in organic source

Organic sources	N	P	K
FYM	0.52	0.25	0.50
Vermicompost	2.00	1.00	1.00
Wheat straw	0.50	0.12	1.25

Result of SPAD showed higher values under inorganic treatments, which might be due to inorganic fertilizers are more preferable than organic ones because they rely on soil organisms to break down first before the nutrient are released. SPAD can be used for estimation of chlorophyll and proxy for N in plant. These results confirmed by Uzik and Zofajova (2000), Srivastava et al. [25], Jan and Boswal [26], Jat et al. [27], Kumar (2015), Sandhu [28] and Praveesh and Shobha [29]. This confirms that

the release of the nitrogen in the soil and the nitrogen supplied with fertilization are the key drivers for the SPAD accuracy to estimate the leaves chlorophyll concentration [30].

3.2 Chlorophyll Content in Leaves at Critical Stages Using Acetone Extraction Method

3.2.1 Chlorophyll 'a'

Data showed Table 3 that the chlorophyll 'a' significantly increased with the application of M_1 over M_2 and M_3 at all stages except M_3 at milking. However, the M_3 was also found significant over M_2 at jointing and milking stage. Maximum chlorophyll 'a' 0.76, 0.83, 1.47 and 0.63 mg g^{-1} leaf tissue with the addition M_1 followed by 0.62, 0.74, 1.21 and 0.57 mg g^{-1} leaf tissue in M_3 and lowest 0.53, 0.63, 0.98 and 0.45 mg g^{-1} leaf tissue in M_2 at CRI, tillering, jointing and milking stages, respectively.

The application of S_4 significantly increased chlorophyll 'a' over S_2 and S_5 at tillering, jointing and milking stage except S_2 at milking stage. The application of S_4 was also found significantly superior to S_3 at jointing stage and at par with remaining stages. However, the S_2 and S_5 were found statistically similar at all stages. The highest chlorophyll 'a' of 0.45, 0.71, 0.84 and 0.59 mg g^{-1} leaf tissue was registered in S_4 followed by S_3 (0.46, 0.68, 0.79 and 0.53 mg g^{-1} leaf tissue) at each stage, respectively.

The interaction effect was found to be significant at jointing and milking stage of wheat. The combination of M_1S_4 was recorded the highest (1.80 and 0.73 mg g^{-1} leaf tissue) chlorophyll 'a' which was significantly superior to all the combination and at par with M_3S_4 (1.59 mg g^{-1} leaf tissue) at jointing stage and M_1S_3, M_1S_2, M_3S_3 (0.69, 0.66 and 0.68 mg g^{-1} leaf tissue) at CRI stage. However, the lowest value found under M_2S_4 (0.53 mg g^{-1} leaf tissue) at CRI, M_2S_2 (0.60 and 0.38 mg g^{-1} leaf tissue) at tillering and milking and M_2S_5 (0.83 mg g^{-1} leaf tissue) at jointing stage.

3.2.2 Chlorophyll 'b'

It can be seen from the data Table 4 that the chlorophyll 'b' significantly increased with the application of M_1 over M_2 at all stages. However, the M_1 was also found significant over M_2 at tillering and milking stage and at par at CRI and jointing stage. The M_3 was also found significant over M_2 at jointing stage. Maximum chlorophyll 'b' 0.44, 0.78, 0.87 and 0.57 mg g^{-1} leaf tissue with the addition M_1 followed by 0.37, 0.52, 0.74 and 0.44 mg g^{-1} leaf tissue in M_3 and lowest 0.32, 0.46, 0.57 and 0.40 mg g^{-1} leaf tissue in M_2 at CRI, tillering, jointing and milking stages, respectively.

The application of S_3 and S_4 significantly increased chlorophyll 'b' over S_2 and S_5 at each stage except S_3 at jointing. However, the S_4 was also found significantly superior to S_3 at milking stage and at par with remaining stages. The S_2 and S_5 were found statistically similar at all stages. The highest chlorophyll 'b'of 0.45, 0.71, 0.84 and 0.59 mg g^{-1} leaf tissue was registered in S_4 followed by S_3 (0.46, 0.68, 0.79 and 0.53 mg g^{-1} leaf tissue) at each stage, respectively.

The interaction effect was found to be significant for all the stage of wheat. The combination of M_1S_4 (0.51, 0.99, 1.05 and 0.78 mg g^{-1} leaf tissue) was recorded the highest chlorophyll 'a', which was significantly superior to all the combination and at par with M_1S_3 (0.57, 0.85 and 0.91 mg g^{-1}) leaf tissue at CRI, tillering and milking stage, respectively and M_1S_5 (0.39 mg g^{-1} at CRI). However, the lowest value found under M_2S_5 of 0.31, 0.43, 0.52 and 0.32 mg g^{-1} leaf tissue at each stage, respectively.

3.2.3 Total chlorophyll

Data indicated Table 5 that the total chlorophyll significantly increased with the application of M_1 over M_2 and M_3 at all the growth stages. However, the M_3 was also found significant over M_2 at all the growth stages except at CRI. Maximum total chlorophyll 1.19, 1.64, 2.25 and 1.14 mg g^{-1} leaf tissue with the addition M_1 followed by 0.95, 1.42, 1.91 and 0.97 mg g^{-1} leaf tissue in M_3 and lowest 0.90, 1.11, 1.53 and 0.84 mg g^{-1} leaf tissue in M_2 at CRI, tillering, jointing and milking stages, respectively.

The application of S_4 significantly increased total chlorophyll over S_2 and S_5 at each stage except S_5 at CRI. However, the S_4 was also found significantly superior toS_3 at jointing stage and at par with remaining stages. The application of S_3 was found significant over S_2 at CRI and milking and over S_5 at tillering, jointing and milking stage.The S_2 was found significant over S_5 at jointing stage and at par with remaining stages. The highest total chlorophyll of 1.14, 1.55. 2.33 and 1.10 mg g^{-1} leaf tissue was registered in S_4 followed by S_3 (1.16, 1.51, 2.04 and 1.09 mg g^{-1} leaf tissue) at each stage, respectively.

Table 2. Effect of different sources of nutrients and levels of NPK on SPAD reading at different growth stage

M/S	CRI				Tillering				Jointing				Milking			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
S_1	3.74	3.85	3.76	3.78	3.62	4.98	3.73	4.11	14.66	14.82	13.69	14.39	15.88	14.83	16.39	15.70
S_2	8.80	4.89	7.20	6.96	17.07	6.02	8.97	10.68	23.24	17.22	21.41	20.62	31.32	17.49	22.52	23.78
S_3	11.60	5.02	8.35	8.32	18.85	6.29	12.53	12.56	27.52	16.54	22.05	22.04	32.22	19.92	27.25	26.46
S_4	12.33	4.93	8.86	8.71	19.25	6.27	11.06	12.19	27.97	17.06	22.27	22.44	37.93	15.94	28.38	27.42
S_5	11.63	3.02	6.07	6.90	18.90	5.69	9.77	11.45	25.46	13.63	21.95	20.35	31.80	16.46	22.67	23.64
Mean	9.62	4.34	6.85	6.94	15.54	5.85	9.21	10.20	23.77	15.85	20.27	19.97	29.83	16.93	23.44	23.40
SEm±	0.215				0.337				0.589				0.772			
CD(P=0.05)	1.306				2.050				3.581				4.695			
SEm±	0.327				0.447				0.825				0.824			
CD(P=0.05)	1.066				1.454				2.688				2.682			
Int I	0.567				0.774				1.430				1.427			
CD(P=0.05)	1.611				2.199				4.064				4.055			
Int II	0.665				0.966				1.739				2.004			
CD(P=0.05)	1.889				2.747				4.942				5.695			

SEm±- Standard error mean, CD -Critical difference, M_1- Inorganic, M_2- Organic, M_3- INM (50% inorganic+ 50% organic), Levels of NPK kg ha^{-1}- S_1- Control, S_2- 100% NPK (120-60-40), S_3- 150% NPK (180-90-60), S_4- 200% NPK(240-120-80), S_5- STV based NPK (149-176-33), Interaction- I-For comparison of two NPK levels at the same source of nutrients, Interaction- II-For comparison of two sources of nutrient at same or different NPK levels

Table 3. Effect of different sources of nutrients and levels of NPK on chlorophyll a (mg g^{-1} leaf tissue) at different growth stage

M/S	CRI				Tillering				Jointing				Milking			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
S_1	0.53	0.40	0.50	0.47	0.60	0.59	0.62	0.61	1.11	0.96	0.90	0.99	0.52	0.44	0.52	0.50
S_2	0.71	0.55	0.60	0.62	0.82	0.60	0.70	0.71	1.38	1.17	1.21	1.25	0.66	0.38	0.64	0.56
S_3	0.88	0.62	0.68	0.73	0.92	0.65	0.78	0.79	1.53	1.00	1.32	1.28	0.69	0.48	0.68	0.62
S_4	0.87	0.53	0.72	0.70	0.99	0.69	0.86	0.84	1.80	0.97	1.59	1.45	0.73	0.50	0.53	0.58
S_5	0.82	0.54	0.62	0.66	0.82	0.63	0.76	0.74	1.55	0.83	1.01	1.13	0.56	0.45	0.49	0.50
Mean	0.76	0.53	0.62	0.64	0.83	0.63	0.74	0.74	1.47	0.98	1.21	1.22	0.63	0.45	0.57	0.55
SEm±	0.022				0.025				0.025				0.016			
CD(p=0.05)	0.132				0.149				0.150				0.096			
SEm±	0.026				0.027				0.046				0.020			
CD(p=0.05)	0.083				0.089				0.148				0.067			
Int I	0.044				0.048				0.079				0.035			
CD(P=0.05)	NS				NS				0.224				0.101			
Int II	0.059				0.065				0.086				0.045			
CD(P=0.05)	NS				NS				0.245				0.127			

Table 4. Effect of different sources of nutrients and levels of NPK on chlorophyll "b" (mg g^{-1} leaf tissue) at different growth stage

M/S	CRI				Tillering				Jointing				Milking			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
S_1	0.24	0.28	0.26	0.26	0.57	0.32	0.37	0.42	0.62	0.49	0.49	0.54	0.39	0.28	0.35	0.34
S_2	0.38	0.31	0.33	0.34	0.68	0.47	0.53	0.56	0.87	0.54	0.83	0.74	0.53	0.39	0.47	0.46
S_3	0.57	0.33	0.48	0.46	0.85	0.55	0.63	0.68	0.91	0.61	0.85	0.79	0.62	0.41	0.56	0.53
S_4	0.51	0.37	0.48	0.45	0.99	0.52	0.63	0.71	1.05	0.67	0.80	0.84	0.78	0.60	0.40	0.59
S_5	0.48	0.31	0.32	0.37	0.83	0.43	0.42	0.56	0.89	0.52	0.75	0.72	0.55	0.32	0.41	0.43
Mean	0.44	0.32	0.37	0.38	0.78	0.46	0.52	0.59	0.87	0.57	0.74	0.73	0.57	0.40	0.44	0.47
SEm±	0.014				0.020				0.024				0.017			
CD(p=0.05)	0.082				0.119				0.144				0.105			
SEm±	0.019				0.019				0.024				0.016			
CD(p=0.05)	0.063				0.062				0.078				0.053			
Int I	0.034				0.033				0.041				0.028			
CD(P=0.05)	0.095				0.094				0.118				0.080			
Int II	0.040				0.049				0.060				0.043			
CD(P=0.05)	0.115				0.140				0.171				0.122			

Table 5. Effect of different sources of nutrients and levels of NPK on total chlorophyll (mg g^{-1} leaf tissue) at different growth stage

M/S	CRI				Tillering				Jointing				Milking			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
S_1	0.73	0.75	0.73	0.74	1.17	0.96	1.06	1.07	1.53	1.45	1.37	1.45	0.85	0.74	0.84	0.81
S_2	1.05	0.93	0.92	0.97	1.67	1.12	1.43	1.41	2.06	1.76	1.94	1.92	1.10	0.78	1.04	0.97
S_3	1.41	0.98	1.10	1.16	1.83	1.18	1.52	1.51	2.39	1.56	2.19	2.04	1.28	0.86	1.14	1.09
S_4	1.36	0.94	1.10	1.14	1.93	1.15	1.57	1.55	2.84	1.59	2.57	2.33	1.33	1.01	0.97	1.10
S_5	1.40	0.88	0.89	1.06	1.59	1.13	1.50	1.40	2.43	1.31	1.48	1.74	1.14	0.79	0.85	0.92
Mean	1.19	0.90	0.95	1.01	1.64	1.11	1.42	1.39	2.25	1.53	1.91	1.90	1.14	0.84	0.97	0.98
SEm±	0.026				0.026				0.041				0.019			
CD(P=0.05)	0.156				0.155				0.251				0.118			
SEm±	0.031				0.032				0.048				0.027			
CD(P=0.05)	0.101				0.104				0.155				0.086			
Int I	0.054				0.055				0.082				0.046			
CD(P=0.05)	0.153				0.158				0.234				0.131			
Int II	0.070				0.071				0.111				0.057			
CD(P=0.05)	0.200				0.202				0.314				0.161			

The interaction effect were found to be significant for all the stage of wheat. The combination of M_1S_4 (1.36, 1.93, 2.84 and 1.33 mg g^{-1} leaf tissue) was recorded the highest total chlorophyll which was significantly superior to all the combination and at par with M_1S_3 (1.41, 1.83, 1.28 mg g^{-1} leaf tissue) at CRI, tillering and milking, M_1S_5 (1.40 mg g^{-1} leaf tissue) at CRI, and M_3S_4 (2.57 mg g^{-1} leaf tissue) at jointing. However, the lowest value found under M_2S_5 (0.88, 1.31 mg g^{-1} leaf tissue) at CRI and jointing and M_2S_2 (1.12 and 0.78 mg g^{-1} leaf tissue) at tillering and milking stage.

Maximum chlorophyll content ('a', 'b' and total) in inorganic fertilizers may be due to inorganic fertilizers release nutrient rapidly particularly N which makes them easily available to plants at early growth. Nitrogen is a structural element of chlorophyll and protein molecules, and it thereby affects formation of chloroplasts and accumulation of chlorophyll in them [31]. The influence of phosphorus on formation of green pigments in the leaf depends primarily on its concentration (Bojovic and Stojanovic, 2006). K is closely associated with process of photosynthesis and transport of photosynthate to storage organs, responsible for maintaining proper water potential, turgid pressure and promoting cell elongation in the leaves which resulted higher chlorophyll content. Our results also confirmed by Chinthapalli et al. [32], Azab [33], Mohammadi et al. [34] and Saharan et al. [35] and Filho et al. [36].

3.3 Correlation between SPAD and Chlorophyll Content

Data given in Table 6and Fig 1, 2, and 3 indicated that the SPAD value had a positive correlation with chlorophyll 'a' (0.98, 0.92, 0.93 and 0.88) at different growth stages. A significant and positive correlation was found between SPAD value and chlorophyll 'b' (0.93, 0.94, 0.99 and 0.92) at all the growth stage. Similarly SPAD value also presented a strong correlation with total chlorophyll (0.94, 0.97, 0.93 and 0.97) at all the growth stages. SPAD value was having strong linear relationship with chlorophyll 'a' having coefficient of determination 0.89, 0.85, 0.79 and 0.93 respectively. Coefficient of determination values for SPAD showed a good linear relationship with chlorophyll 'b' having values 0.83, 0.78, 0.91 and 0.60 respectively.

Coefficient determination of values between SPAD value and total chlorophyll showed linear relationship having values 0.85, 0.87, 0.78 0.77 at CRI, tillering, jointing and milking stages, respectively. We can consider the SPAD measuring device as efficient to evaluate the actual amount of these pigments. These results are in accordance with other studies. According to Piekielek et al. [37] and Dwyer, Tollenaar, and Houwing [15] this indirect evaluation of chlorophyll content in the leaf can be used to predict the nutritional N level in plants, because the correlation with the amount of pigment was positive in relation to N concentration.

Table 6. Mean, correlation and coefficient of determination between SPAD values and chlorophyll content at each growth stage (n=15)

Characters	CRI	Tillering	Jointing	Milking
SPAD mean	6.94	10.20	19.97	23.40
r	0.98**	0.92**	0.93**	0.88*
Chlorophyll " a" mean	0.64	0.74	1.22	0.55
Simple regression equation	0.0422 (SPAD) + 0.3447	0.02 (SPAD) + 0.5324	0.053 (SPAD) + 0.163	0.168(SPAD) + 0.615
R^2 value	0.89	0.85	0.79	0.93
Chlorophyll "b" mean	0.38	0.59	0.73	0.47
r	0.93**	0.94**	0.99**	0.92**
Simple regression equation	0.0294 (SPAD) + 0.1719	0.0288 (SPAD) + 0.2926	0.0338 (SPAD) + 0.0509	0.0139 (SPAD) + 0.143
R^2 value	0.83	0.78	0.91	0.60
Chlorophyll "total" mean	1.01	1.39	1.90	0.98
r	0.94**	0.97**	0.93**	0.97**
Simple regression equation	0.0679 (SPAD) + 0.5409	0.0482 (SPAD) + 0.897	0.0879 (SPAD) + 0.1423	0.0219 (SPAD) + 0.4679
R^2 value	0.88	0.87	0.78	0.77

***. Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level*

Fig Relationship of Chlorophyll with SPAD values at each growth stage (n=15)

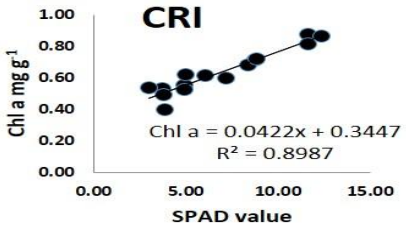

Fig. 1 (a)

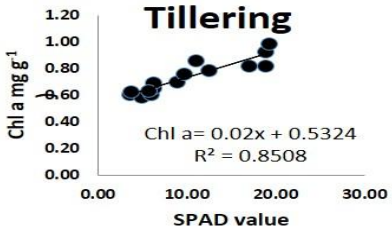

Fig. 1 (b)

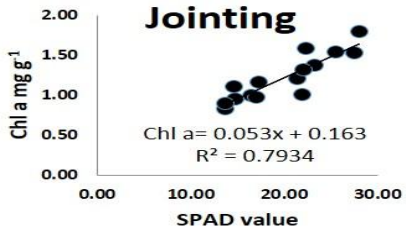

Fig. 1 (c)

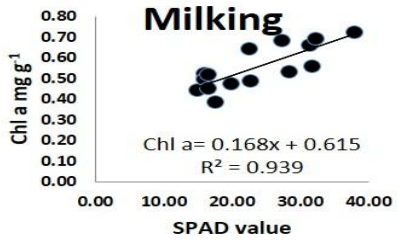

Fig.1 (d)

Fig. 1. Relationship of Chlorophyll "a" with SPAD values at each growth stage

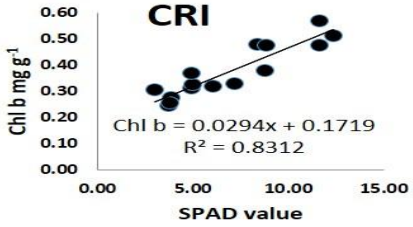

Fig. 2 (a)

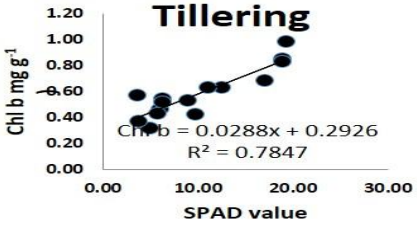

Fig. 2 (b)

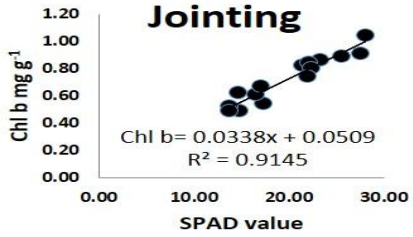

Fig. 2 (c)

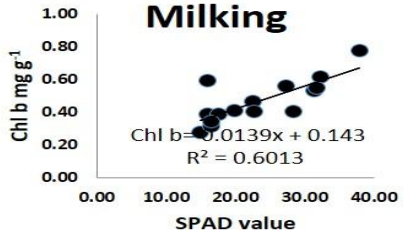

Fig. 2 (d)

Fig. 2. Relationship of Chlorophyll "b" with SPAD values at each growth stage

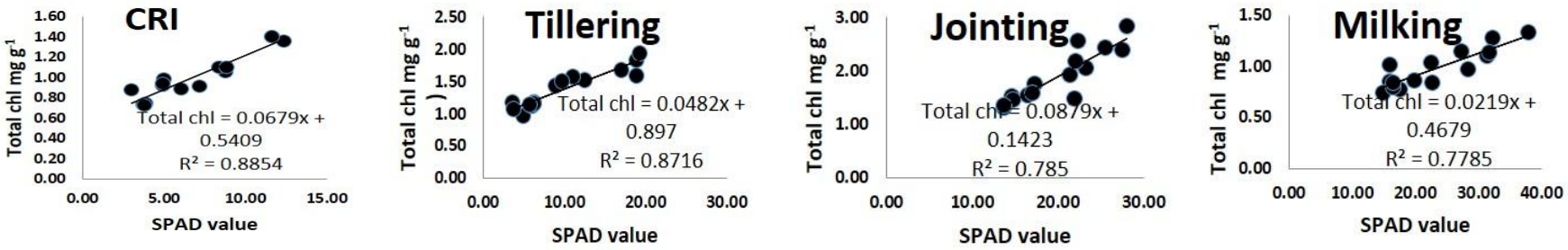

Fig. 3 (a) **Fig. 3 (b)** **Fig. 3 (c)** **Fig. 3 (d)**

Fig. 3. Relationship of Chlorophyll "total" with SPAD values at each growth stage

There is a strong positive relationship between the SPAD and N concentration in the leaves of the plants, although this is more evident in the later growth stages [14], and there is also a high correlation of SPAD with chlorophyll content [15-16].

4. CONCLUSIONS

The results revealed that inorganic sourcesof nutrients showed significant increase in the SPAD readings and chlorophyll content in leaves and over organics at all the stages. All the levels of nutrient were significantly increased but 150% and 200% NPK showed its superior to 100% NPK for SPAD (at CRI and tillering), chlorophyll a (at CRI), chlorophyll b (at CRI, tillering and milking), respectively. Interaction showed that the application of 150 and 200% NPK with inorganic sources were found significant over the same level of NPK with integrated source of nutrients for total chlorophyll content and SPAD value at all growth stages except 150% NPK for total chlorophyll at jointing and milking stage and SPAD value at milking stage. The correlation between SPAD value and chlorophyll 'a', 'b' and total were found significantly and positively and coefficient of determination values showed linear relationship at all the growth stage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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