



## Effect of EMS on morpho-physiological characters of wheat in reference to stay green trait

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**Abstract:** To feed the ever growing world population, the demand of food supply must be increased by 70 % of major cereal crops like wheat, rice etc. It was predicted that the detrimental effect of abiotic stresses like drought, heat, salt etc. on yield would be decreased by genetic improvement in terms of photosynthetic response, long green leaf duration and delayed leaf senescence. 'Stay green' is a vital trait of all crops which is directly associated with the capacity of the plant to maintain CO<sub>2</sub> assimilation, photosynthesis and chlorophyll content. The present study was conducted to develop the stay green mutants genotype by using 1.5 % Ethyl Methane Sulphonate (EMS). The variety K 7410 showed highest leaf area 37.34 cm<sup>2</sup>, seeds per spike 65.47, 1000 grain weight 62.03 g after treatment of EMS among morphological characters observed. Among physiological characters of wheat variety Sonalik showed lowest RWC (21.48 %), HD 2135 showed lowest chlorophyll content (33.53 µg/cm<sup>2</sup>) and C 306 showed lowest photosynthetic rate (15.05 µmol/m<sup>2</sup>sec) after treatment of EMS. But varieties K 7410, VL 401 and RAJ 3765 varieties showed higher value of physiological characters after the treatment. The results suggested that the stay green trait had been developed by mutation (EMS) in these three wheat varieties and they can exhibit better tolerance under abiotic stress conditions like drought, high temperature. Such results would prove useful for further research and crop management in stress affected areas or under unfavourable climatic conditions.

**Keywords:** Chlorophyll, Morphological, Photosynthesis, RWC, Stay green, Wheat

### INTRODUCTION

Today, food security has become a major challenge for all countries to feed up their rapid growing population, so world food supply must be increase by about 70 % by 2050 (Anon, 2009). In recent years, wheat grain requirements of developing countries have increased with 2 % (Donmez *et al.*, 2001; Curtis, 2002). A significant increase in crop productivity will be required to achieve this target using limited crop-growing areas which is also the major drawback (Parry *et al.*, 2011; Reynolds *et al.*, 2011). Wheat production is highly sensitive to climatic and environmental variations due to global warming (Porter and Semenov, 2005; Sillmann and Roeckner, 2008; Semenov and Shewry, 2011; Lobell, *et al.*, 2012). Increasing temperature and incidence of drought associated with global warming are posing serious threats to food security (Bhullar and Jenner, 1985; Fischer, 1986; Lobell *et al.*, 2013) and challenge to plant breeders and crop scientists who have limited time and resources for improvement in crop production rate (Semenov and Halford, 2009; Foulkes *et al.*, 2011; Zheng *et al.*, 2012).

In recent years, researchers show that some physiological criteria such as stomatal conductance (Bahar *et al.*, 2009), photosynthetic rate (Koc *et al.*, 2003), membran thermal stability (Yıldırım *et al.*, 2009), canopy tem-

perature depression (Bahar *et al.*, 2008), delayed leaf senescence Gregersen *et al.* (2008) and chlorophyll content (Yıldırım *et al.*, 2011) provide a gain on wheat production and may be helpful in abiotic stress majorly heat, drought and salt stress tolerance (Reynolds *et al.*, 2001; Balota *et al.*, 2007). Stay green trait induces many morpho-physiological, biochemical and molecular response on plants; so that plants are able to develop tolerance mechanisms which will provide to be adapted to limited environmental conditions (Arora *et al.*, 2002; Bohnert *et al.*, 2006; Shinozaki and Yamaguchi, 2007; Gholamin *et al.*, 2010). Stay green phenotypes maintain green leaf area for a longer period (Spano *et al.*, 2003), maintain CO<sub>2</sub> assimilation (Hafsi *et al.*, 2007) under stress conditions. Total flag leaf photosynthesis, chlorophyll content, the onset of senescence and green leaf duration have all been found to be positively correlated with wheat grain yield (Richards, 2000; Kichey *et al.*, 2007; Wang *et al.*, 2008; Gaju *et al.*, 2011). Stay-green describes the delayed senescence during post-anthesis stages of plant development (Thomas and Howarth, 2000) and genetic variation for this trait has been reported in wheat (Hafsi *et al.*, 2007; Falqueto *et al.*, 2009; Srivalli and Khanna-Chopra, 2009). Thomas and Howarth, (2000), described five types of stay-green phenotypes: Type A phenotypes shown late initiation of senescence with a normal

senescence rate. Type B phenotypes show normal initiation of senescence with a slower rate of senescence. Type C phenotype shows lesion in chlorophyll degradation, leaving the rest of the senescence process unaffected. Type D phenotype shows rapid death (freeze, boil, dry) ensures maintenance of leaf colour in dead leaf. Type E: enhanced greenness but unchanged initiation and rate of senescence. As a result, the overall process of senescence will take longer to complete. Types A, B, and possibly E are functionally stay-green: they maintain photosynthetic capacity in their green tissues. Therefore they may be a potential means to improve grain yield. Crops with 'stay green' phenotype such as sorghum has been reported to have increased yield in water limited conditions because they are able to keep their stalk transporting system functioning under severe drought conditions (Xu *et al.*, 2000). Many 'stay green' mutants, referred to as functional stay green or non-yellowing in various plant species have been reported to maintain leaf greenness after the grain-ripening stage and give better yield (Fang *et al.*, 1998; Spano *et al.*, 2003). Therefore, this work was aimed to bring light on the most important trait i.e. Stay-Green trait and its major role in improvement in morphological, physiological and biochemical characters for high yield production of wheat under stressed environment.

## MATERIALS AND METHODS

The present research work was carried out at laboratory of Department of Biotechnology, College of Agriculture, of S. V. P. University of Agriculture and Technology, Meerut during rabi season. A total of ten varieties of wheat cultivars viz., HUW 510, C 306, Sonalika, HD 2135, HD 2177, VL 401, K 9162, RAJ 3765, K 68, K 7410 were collected to study the effect of EMS treatment on morpho-physiological and biochemical characters. About 30 seeds of all wheat varieties were soaked in EMS solution (1.5 % in distilled water) for 90 min in petri plates. Thereafter, the treated seeds of wheat were sown in pots for further collection of data.

**Morphological evaluation:** The data was observed by randomly selecting five plants from each variety. The data was observed like Plant height (cm), Number of tillers, Leaf Area (of flag leaf), Days of Maturity (days from sowing to the 98-100 % loss of total chlorophyll content of plant, Length of spike (cm), Seeds per Spike (Mean of seeds counted from 5 randomly sampled spikes at maturity is recorded for analysis), Thousand grain weight (in gm.) was recorded from control and treatment plants for comparative study.

**Physiological evaluation:** After inducing mutation by EMS for developing stay green trait the data for Related Water Content (RWC), Chlorophyll content and Photosynthetic rate was recorded. The RWC was measured on the wheat seedling organs following the

method of Turner, (1981). Fresh weight (FW) of flag leaf was determined immediately after harvest, and then tissue were allowed to float in distilled water until fully rehydrated. The wheat organs were weighted for turgid weight (TW). The turgid organs were dried in a hot oven at 80 °C to constant weight, and dry weight (DW) was recorded. The RWC was calculated as:  $RW (\%) = (FW - DW) / (TW - DW) \times 100$ .

Chlorophyll meter (SPAD 520) were used to measure the relative chlorophyll content ( $\mu\text{gm}/\text{cm}^2$ ) of the leaves and Infra Red Gas Analyzer (IRGA) was used to measure the photosynthetic rate of leaves ( $\mu\text{mol}/\text{m}^2\text{sec}$ ). Five readings were taken from five plant leaves of same variety and their average was considered for determination of RWC, Chlorophyll content and Photosynthetic rate

**Statistical analysis:** The experimental data obtained from randomly selected five plants from each replicates were subjected to the statistical analysis outlined by Panse and Sukhatme, (1978). The significance of differences among treatment means was tested by 'F' test and critical differentiation (at 5 per cent probability) was calculated by the method given by Pearson K. (1895).

## RESULTS AND DISCUSSION

**Morphological characterization:** In the present study, plant height was recorded at final stage of maturity. The plant height was found to be increased after the treatment of EMS (Table 1, Fig. 1). In control plants, height of plant was varied from 96.15 cm to 81.57 cm. After treatment Sonalika showed highest plant height 98.83 cm whereas variety K 7410, RAJ 3765 and VL 401 showed decrement in plant height. The number of productive tillers per plant was varied from 9.58 in K 7410 to 7.47 in HD 2177 in controlled plants. The number of productive tillers decreases after all treatments except in K 7410, RAJ 3765 and VL 401 variety. Mean value of flag leaf area was varied from 34.17  $\text{cm}^2$  to 24.03  $\text{cm}^2$  in variety K 7410 and C 306 in control plants (Table 1, Fig. 1). Total leaf area of flag leaf of wheat varieties was reduced significantly after EMS treatment except in variety HUW 510, VL 401, K 7410 and RAJ 3765 were found to be increased after the EMS treatment. Leaf area of flag leaf is directly related to higher photosynthesis and high chlorophyll content. Genotypes possessing the ability to maintain green leaf area duration (stay green traits) throughout grain filling are potential candidates to assure better yield (Hoang and Kobata, 2009; Larbi and Mekliche, 2004) similarly supporting results were obtained in present study also. After EMS treatment, variety K 7410, VL 401 and RAJ 3765 took lesser time to get mature after EMS treatment comparatively with others so, it could be considered as better genotype and other showed increment compared to control (Table 1, Fig. 1). The length of spike varied from 12.33cm in K

Table 1. Morphological characters of wheat.

Variety	Plant height (cm)				No. of tillers				Leaf area(cm <sup>2</sup> )				Days to maturity			
	Control		Treatment (1.5% EMS)		Control		Treatment (1.5% EMS)		Control		Treatment (1.5% EMS)		Control		Treatment (1.5% EMS)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
HUW 510	86.33	±2.3	95.00	±1.1	9.37	±0.4	8.17	±0.1	29.80	±0.4	31.07	±0.1	143	±0.0	145	±0.0
C 306	87.25	±0.4	97.42	±1.6	8.23	±0.3	6.20	±0.8	24.03	±0.0	22.08	±0.2	143	±0.0	146	±0.0
Sonalika	96.15	±0.5	98.83	±1.8	7.58	±0.4	6.00	±0.6	25.13	±0.1	22.63	±0.2	136	±0.0	140	±0.0
HD 2135	86.67	±2.4	92.67	±1.6	8.17	±0.3	6.67	±0.7	26.00	±0.2	23.17	±0.2	145	±0.0	147	±0.0
HD 2177	81.57	±0.2	90.75	±1.5	7.47	±0.2	7.17	±0.9	25.01	±0.2	22.26	±0.3	140	±0.0	142	±0.0
VL 401	90.17	±0.6	85.08	±1.8	9.30	±0.2	10.22	±0.8	32.39	±0.0	35.34	±0.2	139	±0.0	134	±0.0
K 9162	89.13	±0.5	94.42	±0.9	8.52	±0.3	7.13	±1.2	25.03	±0.0	21.60	±0.2	143	±0.0	146	±0.0
RAJ 3765	96.15	±0.5	82.90	±1.3	9.37	±0.1	10.41	±0.3	32.47	±1.4	35.85	±0.6	139	±0.0	134	±0.0
K 68	88.98	±0.6	97.50	±1.2	8.42	±0.3	6.50	±0.5	30.03	±0.2	26.83	±0.4	141	±0.0	144	±0.0
K 7410	86.67	2.4	83.17	±1.3	9.58	±0.2	10.55	±0.4	34.17	±0.1	37.34	±0.2	133	±1.4	130	±0.4
SEm±	0.34				0.28				0.21				0.01			
CD (P=0.05)	4.233				1.003				1.144				4.895			
Variety	Length of Spike (cm)				Seeds per Spike				1000 Grain Weight(g)							
	Control		Treatment (1.5% EMS)		Control		Treatment (1.5% EMS)		Control		Treatment (1.5% EMS)					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE				
HUW 510	10.30	±0.2	9.13	±0.0	46.33	±0.3	44.73	±0.3	47.50	±0.3	46.29	±0.3				
C 306	10.67	±0.1	9.17	±0.4	49.50	±0.3	45.67	±0.3	42.70	±0.3	39.60	±0.3				
Sonalika	10.90	±0.0	7.23	±0.2	50.33	±0.3	45.33	±0.3	44.00	±0.3	38.40	±0.3				
HD 2135	10.03	±0.0	10.67	±0.2	51.37	±0.3	48.33	±0.3	45.20	±0.3	40.70	±0.3				
HD 2177	9.67	±0.1	8.53	±0.3	45.84	±0.5	39.67	±0.3	46.30	±0.3	41.80	±0.3				
VL 401	10.13	±0.1	12.73	±0.3	59.67	±0.3	63.07	±0.3	51.20	±0.3	60.20	±0.3				
K 9162	10.80	±0.1	11.63	±0.3	50.33	±0.3	44.67	±0.3	47.70	±0.3	43.60	±0.3				
RAJ 3765	12.08	±0.1	12.77	±0.2	62.07	±0.3	63.13	±0.6	56.89	±0.6	60.76	±0.6				
K 68	10.93	±0.0	10.33	±0.3	58.33	±0.3	52.67	±0.3	50.20	±0.3	47.06	±0.3				
K 7410	12.33	±0.0	13.33	±0.0	63.17	±0.3	65.47	±0.3	60.50	±0.3	62.03	±0.3				
SEm±	0.28				0.46											
CD (P=0.05)	0.680				1.206				0.00							

**Table 2.** Physiological characters of wheat.

Variety	RWC(%)				Chlorophyll content ( $\mu\text{g}/\text{cm}^2$ )				Photosynthesis rate ( $\mu\text{mol}/\text{m}^2\text{sec}$ )			
	Control		Treatment (1.5% EMS)		Control		Treatment (1.5% EMS)		Control		Treatment (1.5% EMS)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
HUW 510	60.30	$\pm 0.4$	54.34	$\pm 0.2$	43.55	$\pm 2.2$	40.22	$\pm 1.9$	16.67	$\pm 0.1$	15.53	$\pm 0.1$
C 306	29.43	$\pm 0.2$	24.41	$\pm 0.2$	40.10	$\pm 1.2$	38.57	$\pm 2.1$	16.17	$\pm 0.3$	15.05	$\pm 0.3$
Sonalika	26.29	$\pm 0.1$	21.48	$\pm 0.2$	38.00	$\pm 1.0$	34.72	$\pm 0.8$	18.00	$\pm 0.2$	16.07	$\pm 0.1$
HD 2135	55.19	$\pm 0.1$	52.27	$\pm 0.2$	36.40	$\pm 2.1$	33.53	$\pm 2.2$	17.00	$\pm 0.2$	16.00	$\pm 0.2$
HD 2177	36.37	$\pm 0.1$	32.15	$\pm 0.4$	39.57	$\pm 2.1$	32.72	$\pm 0.8$	18.83	$\pm 0.1$	17.00	$\pm 0.1$
VL 401	58.40	$\pm 0.3$	63.38	$\pm 0.2$	49.50	$\pm 1.5$	51.20	$\pm 1.0$	23.33	$\pm 0.1$	26.13	$\pm 0.1$
K 9162	49.49	$\pm 0.2$	43.34	$\pm 0.2$	39.57	$\pm 2.1$	33.07	$\pm 0.6$	17.70	$\pm 0.1$	16.00	$\pm 0.2$
RAJ 3765	60.69	$\pm 0.1$	63.62	$\pm 0.3$	49.92	$\pm 1.0$	51.92	$\pm 1.4$	23.57	$\pm 0.2$	26.70	$\pm 0.1$
K 68	59.44	$\pm 0.2$	52.23	$\pm 0.3$	39.03	$\pm 2.2$	34.40	$\pm 2.1$	20.13	$\pm 0.1$	18.67	$\pm 0.3$
K 7410	62.30	$\pm 0.1$	65.77	$\pm 0.1$	50.83	$\pm 1.4$	54.72	$\pm 1.4$	24.03	$\pm 0.1$	28.17	$\pm 0.2$
SEM $\pm$	0.34				0.46				0.32			
CD(P=0.05)	0.739				0.592				0.592			

7410 to 9.67cm in HD 2177 variety in control plants but after treatment of EMS the spike length reduces significantly except in variety K 7410, VL 401 and RAJ 3765 (Table 1, Fig. 2). Seeds per spike are direct measure of yield per plant, hence it is economically important morphological characteristic. The number of seeds per spike was decreasing after EMS treatment in almost all the varieties except in variety K 7410 from (63.17 to 65.47), VL 401 (59.67 to 63.07) and RAJ 3756 (62.07 to 63.13), respectively (Table 1, Fig. 2). 1000 grain weight showed the actual yield of the crop in the field and it varied from 60.50 gms in K 7410 variety to 42.70 gms in C 306 variety in control plants. A significant reduction in 1000 grain weight was observed after treatment of EMS except in variety K 7410, VL 401 and RAJ 3765 (Table 1, Fig. 2). Similarly, Sparkes (2010) suggested that bread wheat senesces faster than both spelt and emmer which could indicate a 'stay green' trait in it. Many researchers also determined the correlation between yield and flag leaf area duration, explaining that delayed senescence holdup remobilization and leads to reduced grain weight (Rawson *et al.*, 1983; Gregersen *et al.*, 2008).

**Physiological characterization:** RWC influences the ability of the plant to recover from stress and consequently affects yield. The RWC in controlled plants varied from 62.30 % to 26.29 % in HUW 510, K 7410 and Sonalika (Table 2, figure 3). The increased RWC after EMS treatment was observed in variety K 7410, VL 401 and RAJ 3765 from 62.30 to 65.77 %, 58.40 % to 63.38 % and 60.69 to 63.62 % and thus could be considered as stay green genotype. Keyvan (2010) reported decreased RWC in wheat plants during induced drought stress which affects the growth and yield as well. Matin, (1990) reported in his study that drought tolerant cultivars (barley) usually maintained higher leaf RWC under the stress. Leaf RWC one of the best growth indices revealing the stress intensity for improving yield in wheat (Gupta *et al.*, 2001). The chlorophyll content is one of the essential parameters which also give direct measure of photosynthesis rate

and can be considered as indicator of stay green genotypes. In present study, chlorophyll content in control plants varied from  $50.83\mu\text{g}/\text{cm}^2$  to  $36.40\mu\text{g}/\text{cm}^2$ . But after treatment of EMS all varieties showed decline in chlorophyll content from control except variety K 7410, VL 401 and RAJ 3765 (Table 2, Fig. 3). So these two varieties had not only maximum survival in stressed environment but also give better yield as well. Similar findings were also reported by Khayatnezhad *et al.* (2011) on *Zeamays*; Shahriari and Khayatnezhad (2011) on wheat and Adinda *et al.* (2012) on wheat and concluded that the genotypes with high leaf chlorophyll content are tolerant to abiotic stress conditions. Chlorophyll content of leaf is indicator of photosynthetic capability of plant tissues (Nageswara *et al.*, 2001) studied on *Arachishypogaea* L. The photosynthesis rate was also found to be increased in K 7410, VL 401 and RAJ 3765 from  $24.03\mu\text{mol}/\text{m}^2\text{sec}$  to  $28.17\mu\text{mol}/\text{m}^2\text{sec}$ ,  $23.33\mu\text{mol}/\text{m}^2\text{sec}$  and  $23.57\mu\text{mol}/\text{m}^2\text{sec}$  to  $26.70\mu\text{mol}/\text{m}^2\text{sec}$ , respectively after the treatment of EMS as chlorophyll content but showed a significant reduction in photosynthetic rate after the treatment in other varieties in comparison to control lowest in C 306 (Table 2, Fig. 3). Such profile of photosynthesis rate indicates that variety K 7410, VL 401 and RAJ 3765 could be considered as stay green mutants. The results are consistent with the findings of Michael *et al.* (2011) on wheat for stay green trait. Extending the duration of photosynthesis is a possible means to increase total photosynthesis, biomass and yield (Richards, 2000). Total flag leaf photosynthesis, chlorophyll content, the onset of senescence (at low nitrogen availability) and green leaf duration directly related with wheat grain yield (Kichey *et al.*, 2007; Wang *et al.*, 2008; Gaju *et al.*, 2011). The findings of Gupta *et al.*, (2001) on wheat also supports the results of the present research work that the stay green trait is directly related to the yield attributes. It means there is a greater scope of using physiological traits coupled with morphological also in selection of better tolerant variety for improving yield in wheat.

## Conclusion

A key factor to increase wheat yield potential is an extended duration of photosynthetic rate and high chlorophyll content. The yield capacity can be increased if the plant would be able to maintain healthy green area index until the end of grain filling ('stay-green'). In water-limited environments such as SL, improvement in drought tolerance which delays leaf senescence will be essential. The present research results supports that the mutated wheat varieties RAJ 3765, VL 401 and K 7410 can perform better in stressed environment such as drought, high treatment etc. These varieties showed high chlorophyll content ( $54.72 \mu\text{g}/\text{cm}^2$ ) and photosynthetic rate ( $28.17 \mu\text{mol}/\text{m}^2\text{sec}$ ) after the treatment of EMS and resulted in high thousand grain weight (62.03g). Hence, the stay green trait can be a vital trait for high yield potential of major cereal crops like wheat, rice etc.

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