

# Evaluation of chlorophyll content as selection criteria for drought tolerance in wheat

Adriana CIULCA<sup>1</sup>, Giancarla VELICEVICI<sup>1</sup>, Mihaela MOATĂR<sup>2</sup>, Camelia TULCAN<sup>2</sup>, Dorin CAMEN<sup>1</sup>, Sorin CIULCA<sup>1\*</sup>

<sup>1</sup> University of Life Sciences "King Mihai I" from Timisoara, Faculty of Engineering and Applied Technologies, Department of Genetic Engineering, e-mail: [adrianaciulca@usvt.ro](mailto:adrianaciulca@usvt.ro), [giancarlavelicevici@usvt.ro](mailto:giancarlavelicevici@usvt.ro), [dorincamen@usvt.ro](mailto:dorincamen@usvt.ro), [sorinciulca@usvt.ro](mailto:sorinciulca@usvt.ro)

<sup>2</sup> University of Life Sciences "King Mihai I" from Timisoara, Faculty of Engineering and Applied Technologies, Department of Forestry, e-mail: [mihaelamoatar@usvt.ro](mailto:mihaelamoatar@usvt.ro), [cameliatulcan@usvt.ro](mailto:cameliatulcan@usvt.ro)

\* Corresponding author: [sorinciulca@usvt.ro](mailto:sorinciulca@usvt.ro)

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## Abstract

Drought and water deficit are two major limiting factors in wheat production. The genotypes having high chlorophyll content express a good photosynthesis rate and ability to tolerate different level of drought. As such, the evaluation of chlorophyll content can be an effective method of selecting tolerant wheat genotypes. The goal of this work was to assess the performance and improvement potential for chlorophyll content in case of 21 F<sub>1</sub> crosses between seven wheat varieties, genetically and ecologically differentiated. For most of the combinations the inheritance of chlorophyll content was controlled by over dominance effects, associated generally with a decrease of this trait. For Fundulea 4 x Apache and Alex x Apache the incomplete dominance leads to a decrease of chlorophyll content. For Xenos x Alex the complete dominance is acting with a negative effect in the inheritance of this trait, while for Fundulea 4 x Xenos the dominance effects are very low. The crosses Fundulea 4 x GKKapos, Fundulea 4 x Alex and Xenos x Turda 2000 showed the highest potential to improve the chlorophyll content in wheat. In this regard, those combinations can be used as genitors or as selection material.

**Keywords:** water stress, photosynthetic pigments, wheat crosses

## Introduction

Under the global climate change the frequency of droughts periods increase, having a negative impact of food security. Water stress is one of the main factors restricting growth, yield and quality of wheat. The mechanisms of drought adaptations in wheat vary in accordance with developmental stage, intensity and duration of stress [11]. Drought affects the growth of wheat throughout the growing season, but during the reproductive and grain-filling phases the highest yield losses are recorded [24].

Chlorophyll is one of the major components of chloroplasts, so that the chlorophyll content is positively correlated with the rate of photosynthesis. Reduction of chlorophyll content under dry conditions is considered a typical symptom of oxidative stress which may be the result of photo-oxidation of pigments and chlorophyll degradation [3].

Under drought stress conditions, the chlorophyll content changes markedly, which can provide information about plant photosynthesis and drought tolerance [25]. Chlorophyll content in wheat varies during drought conditions and can be used as a measure of the photosynthetic ability of plant [11]. Therefore, the reduction of chlorophyll content under water deficit conditions would be caused by the destruction of the chloroplast membrane, excessive swelling and distortion of vesicle blades and the appearance of some lipid droplets [14].

A low concentration of photosynthetic pigments can cause a direct reduction of the photosynthetic potential and implicitly of the yield. Chlorophyll *b*, as the principal component of the photo systems, is more affected than chlorophyll *a* under drought stress. Therefore, genotypes with a low value of ratio for chlorophyll *a/b* show a greater tolerance to water deficit [16]. The decrease of chlorophyll contents under drought is due to a greater activity of chlorophyllase enzyme or to a destruction of thylakoid membrane, with negative impact on photosynthetic performance and yield [22].

Given the strong correlation between leaf colour and chlorophyll content, the change of leaf colour during drought stress is due mainly to chlorophyll loss. Therefore, chlorophyll content was also correlated with

plant biomass and grain yield [26]. The drought tolerant wheat varieties have been found to have superior water use efficiency, high chlorophyll content and dry matter compared to susceptible varieties [10; 21; 23]. Nicolaeva et al. [19] found that the chlorophyll content increased slightly during the first stages of drought but decreased with 13–15% in later period.

According to Bojovic and Markovic [6] there is a very close relation between chlorophyll and nitrogen content in the leaf. As such, SPAD measurement offers the possibility to synchronize N supply with specific crop demand [13]. Even it was found a highly significant positive association between wheat grain yield and SPAD values, this correlation was strong influenced by different factors, like: cultivar, environment and nitrogen content of the soil [17]

The goal of this work was to assess the performance and improvement potential for chlorophyll content for 21 F<sub>1</sub> crosses between seven wheat varieties, genetically and ecologically differentiated.

### Material and Method

The biological material consisted of seven wheat varieties genetically and ecologically differentiated, such as: Fundulea 4, Alex, Apullum, Turda 2000, GKKapos, Apache, Xenos, alongside with their 21 half-diallel crosses, studied using a randomized block design on three replications.

The leaves chlorophyll content was determined using portable chlorophyllmeter SPAD-502 (Konica Minolta), measuring the absorbance at 650 nm, being a non-destructive method [5]. On each flag leaf were performed three readings, the results being expressed in SPAD units.

The estimation of mid (compared to parents mean) and best parent heterosis (compared to the parent with higher value) has been achieved using the formulas proposed by Fonseca and Patterson [9]: Heterosis % =  $[100 * (F_1 - PM)] / PM$ ; Heterobeltiosis =  $[100 * (F_1 - BP)] / BP$ , where; F<sub>1</sub>- performance of hybrid; PM- parents mean; BP- parent with higher value. The type of inheritance was assessed using dominance parameters. In this regard, the potency ratio (PR) was calculated according to dominance ratio ( $d/a$ ), where  $d = (F_1 - MP)$  and  $a = (P_1 - P_2) \frac{1}{2}$ .

The significance of differences between parents and F<sub>1</sub> hybrids were estimated using Multiple Range Test [8]. In order to evaluate the improvement potential of different combinations, the percentage of recombinant lines with a certain level of chlorophyll content was calculated using the method proposed by Hill et al. [12].

### Results and Discussion

According with the data from Table 1, it can be noticed that 77 % of the crosses showed negative values of potency ratio, and a chlorophyll content below the parents mean, respectively. The highest values of potency ratio, associated with a high quantity of chlorophyll have been achieved by the crosses: Fundulea 4 x Turda 2000 (5.39), Fundulea 4 x Alex (5.15) și Fundulea 4 x GKKapos (4.43), combinations at which there were small differences between parents. The lowest values of potency ratio, correlated with a chlorophyll content below the mid parent, were recorded by the crosses: Turda 2000 x Apache (-3.84); GKKapos x Turda 2000 (-3.24) și Xenos x Apullum (-2.52).

Considering the values of potency ratio, we can see that for most of the crosses the inheritance of chlorophyll content was controlled by over dominance effects, associated generally with a decrease of this trait. In the case of Fundulea 4 x Turda 2000, Fundulea 4 x Alex, Fundulea 4 x GKKapos, Xenos x Turda 2000, Xenos x Apache, Fundulea 4 x Apullum and GKKapos x Apullum, the over dominance causes an increase of chlorophyll content. For Fundulea 4 x Apache and Alex x Apache the incomplete dominance leads to a decrease of chlorophyll content. For Xenos x Alex the complete dominance is acting with a negative effect in the inheritance of this trait, while for Fundulea 4 x Xenos the dominance effects are very low. These results are in accordance with the finding of Naroui Rad et al. [18], that the chlorophyll content was mainly controlled by dominant gene action.

**Table 1. Potency ratio for chlorophyll content (SPAD) in wheat crosses**

No	Crosses	PR	No	Crosses	PR
1	Fundulea 4 x Xenos	-0.06 cd	12	GKKapos x Turda 2000	-3.24 hi
2	Fundulea 4 x GKKapos	4.43 a	13	GKKapos x Alex	-2.20 fgh
3	Fundulea 4 x Turda 2000	5.39 a	14	GKKapos x Apache	-2.42 ghi
4	Fundulea 4 x Alex	5.15 a	15	GKKapos x Apullum	1.38 bc
5	Fundulea 4 x Apache	-0.47 de	16	Turda2000 x Alex	-1.63 efg
6	Fundulea 4 x Apullum	1.56 b	17	Turda2000 x Apache	-3.84 i
7	Xenos x GKKapos	-1.52 defg	18	Turda2000 x Apullum	-1.26 defg
8	Xenos x Turda 2000	2.54 b	19	Alex x Apache	-0.78 def

9	Xenos x Alex	-1.06 defg	20	Alex x Apullum	-1.40 defg
10	Xenos x Apache	2.05 b	21	Apache x Apullum	-1.35 defg
11	Xenos x Apullum	-2.52 ghi			

The differences between crosses marked with different letters are significant at p 0.05.  $LSD_{5\%} = 1.49$

For approximately 57 % of the crosses the chlorophyll content was below to both parents, thus displaying a low drought tolerance. Therefore, in the case of two combinations (Fundulea 4 x Xenos; Fundulea 4 x Apache) the chlorophyll content was intermediary to parents. For a third of the crosses, the values of this trait and the tolerance to drought were superior to both parents. Most of the crosses were inferior to parents, showing significant differences to each parent or to parents mean.

**Table 2. Heterosis for chlorophyll content in F<sub>1</sub> wheat crosses**

Crosses	Mid parent heterosis		Best parent heterosis	
	H (%)	Difference	H (%)	Difference
Fundulea 4 x Xenos	117.53 cde	9.09	109.83 efgh	5.45
Fundulea 4 x GKKapos	82.35 h	-9.71	81.68 k	-10.16
Fundulea 4 x Turda 2000	96.76 g	-1.70	91.33 j	-4.81
Fundulea 4 x Alex	73.48 h	-15.44	70.17 l	-18.18
Fundulea 4 x Apache	118.23 cde	9.66	112.97 defg	7.19
Fundulea 4 x Apullum	99.09 g	-0.48	95.22 j	-2.65
Xenos x GKKapos	121.27 cde	10.93	114.19 cde	7.74
Xenos x Turda 2000	81.75 h	-8.89	80.88 k	-9.41
Xenos x Alex	117.78 cde	9.70	105.45 fgh	3.32
Xenos x Apache	107.49 fg	3.70	105.01 gh	2.53
Xenos x Apullum	125.08 abc	12.45	121.48 bcd	10.98
GKKapos x Turda 2000	119.71 cde	10.23	113.86 cdef	7.56
GKKapos x Alex	113.24 ef	7.65	107.30 efgh	4.45
GKKapos x Apache	117.38 cde	9.13	113.03 defg	7.11
GKKapos x Apullum	107.14 fg	3.78	103.78 hi	2.06
Turda2000 x Alex	121.48 cde	11.83	109.80 efgh	5.97
Turda2000 x Apache	134.52 a	17.22	132.81 a	16.57
Turda2000 x Apullum	131.09 ab	15.60	128.66 ab	14.65
Alex x Apache	114.45 def	8.06	104.66 gh	2.84
Alex x Apullum	116.83 de	9.43	107.42 efgh	4.52
Apache x Apullum	123.11 bcd	11.75	122.38 bc	11.44
<i>Experience mean</i>	111.42 <sub>+3.5</sub> 4	5.90 <sub>+1.87</sub>	106.28 <sub>+3.4</sub> 0	3.29 <sub>+1.84</sub>
<i>LSD<sub>5%</sub></i>	9.02	4.77	8.64	4.68

The values of mid parents heterosis range from 73.48 % in Fundulea 4 x Alex to 134.52 % for Turda 2000 x Apache, considering that in the case of six crosses this type of heterosis was over 20 %. Also, the highest mid parent heterosis and heterobeltiosis associated with a high chlorophyll content was recorded in Turda 2000 x Apache, Turda 2000 x Apullum, followed by the crosses Apache x Apullum and Xenos x Apullum. In the case of following crosses: Fundulea 4 x GKKapos, Xenos x Turda 2000, Fundulea 4 x Alex, negative values for both type of heterosis were recorded, associated with low chlorophyll content and tolerance to drought.

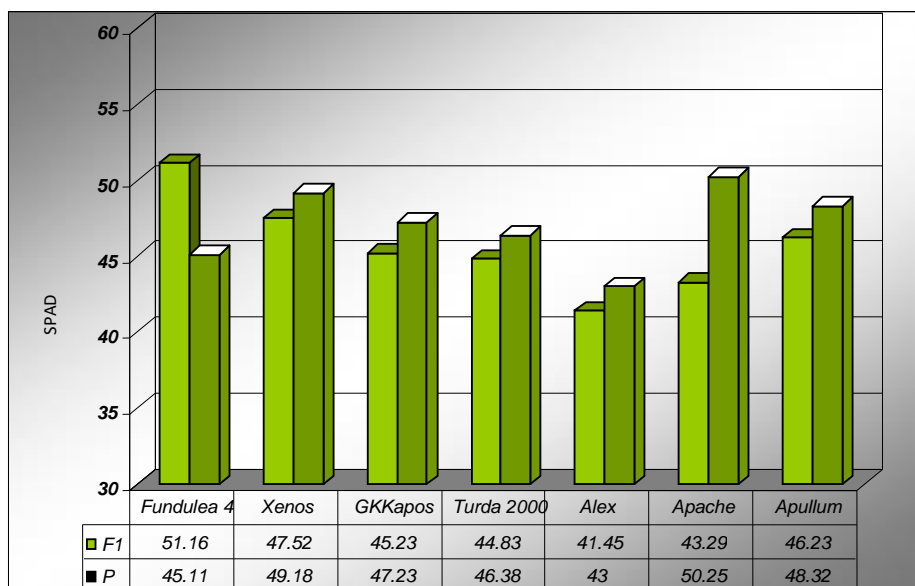
**Table 3. The comparisons between F<sub>1</sub> crosses groups with the same recurrent parent concerning chlorophyll content (SPAD)**

Recurrent parent	Average	Fundulea 4	Xenos	GKKapos	Turda 2000	Alex	Apache	Apullum
Fundulea 4	51.16		3.64	5.93	6.33	9.71**	7.87*	4.93
Xenos	47.52			2.29	2.69	6.07	4.23	1.29
GKKapos	45.23				0.40	3.78	1.94	-1.00
Turda 2000	44.83					3.38	1.54	-1.40
Alex	41.45						-1.84	4.78
Apache	43.29							-2.94
Apullum	46.23							

LSD	LSD	LSD <sub>0,1</sub>
5%	1%	%
7,17	9,63	12,69

\*Significant at p<0.05; \*\*Significant at p<0.01

Concerning the groups of crosses for each parent (Table 3), it was noticed an amplitude of 9.71 ranging from 41.45 for the crosses group of Alex variety and 51.16 for the crosses group of Fundulea 4 variety. On the background of this low variability there are significant differences between the group of Fundulea 4 which exhibit higher chlorophyll content to the crosses of Apache and Alex varieties. According to the data from Figure 1 it was noted that most of the hybrids exhibited lower chlorophyll content to the recurrent parent, except for the hybrids of Fundulea 4 variety which recorded an increase of 6.05 for chlorophyll content.



**Figure 1. Chlorophyll content (SPAD) for F<sub>1</sub> crosses groups with the same recurrent parent**

The highest improvement potential of chlorophyll content was noticed in Fundulea 4 x GKKapos which enables the selection of 46.41 % recombinant lines with up to 56 SPAD units. The achievement of this target in a proportion of 95% it is necessary to select minimum 5 lines, or 11 lines for 99.9% accuracy, respectively (table 4).

The crosses Fundulea 4 x Alex, Xenos x Turda 2000, also have a high potential to obtain approx. 38.97-40.13 % recombinant lines with an average value of this indicator of at least 56 SPAD. In order to achieve this target with 95 % accuracy, it is necessary to select at least six lines or 13-14 lines for a success rate of 99.9 %. For Fundulea 4 x Turda 2000 and Xenos x Apache combinations, the probability to select lines with a chlorophyll content of at least 56 SPAD is 24.83%, in terms of extracting 10-24 lines for a success rate of 95-

99.9 %. The combinations Fundulea 4 x Apullum, has a breeding potential of 12.1% to achieve a chlorophyll content of 56 SPAD, by extracting 23-54 lines.

**Table 4. The improvement potential of crosses in terms of the percent of recombinant lines with a chlorophyll content above 56 SPAD and the necessary number of selected lines**

Crosses	$F_1$ (SPAD)	<i>s</i>	<i>LR</i> %	$n_{5\%}$	$n_{1\%}$	$n_{0,1\%}$
Fundulea 4 x Xenos	46.92	1.86	0.01	29956	46049	69074
Fundulea 4 x GKKapos	55.52	5.43	46.41	5	7	11
Fundulea 4 x Turda 2000	52.57	5.01	24.83	10	16	24
Fundulea 4 x Alex	54.92	4.29	40.13	6	9	13
Fundulea 4 x Apache	45.29	3.57	0.13	2303	3540	5310
Fundulea 4 x Apullum	51.72	3.67	12.10	23	36	54
Xenos x GKKapos	45.24	0.93	0.01	29956	46049	69074
Xenos x Turda 2000	54.88	4.04	38.97	6	9	14
Xenos x Alex	39.55	4.36	0.01	29956	46049	69074
Xenos x Apache	51.92	6.03	24.83	10	16	24
Xenos x Apullum	46.59	2.41	0.01	29956	46049	69074
GKKapos x Turda 2000	44.06	3.24	0.01	29956	46049	69074
GKKapos x Alex	35.84	2.06	0.01	29956	46049	69074
GKKapos x Apache	41.42	6.37	1.10	271	416	625
GKKapos x Apullum	49.29	5.16	9.68	29	45	68
Turda2000 x Alex	39.20	4.02	0.01	29956	46049	69074
Turda2000 x Apache	33.46	3.83	0.01	29956	46049	69074
Turda2000 x Apullum	44.90	2.85	0.01	29956	46049	69074
Alex x Apache	40.99	2.09	0.01	29956	46049	69074
Alex x Apullum	38.22	3.42	0.01	29956	46049	69074
Apache x Apullum	46.67	4.51	1.92	155	238	356

The crosses Fundulea 4 x Alex, Xenos x Turda 2000, also have a high potential to obtain approx. 38.97-40.13 % recombinant lines with an average value of this indicator of at least 56 SPAD. In order to achieve this target with 95 % accuracy, it is necessary to select at least six lines or 13-14 lines for a success rate of 99.9 %. For Fundulea 4 x Turda 2000 and Xenos x Apache combinations, the probability to select lines with a chlorophyll content of at least 56 SPAD is 24.83%, in terms of extracting 10-24 lines for a success rate of 95-99.9 %. The combinations Fundulea 4 x Apullum, has a breeding potential of 12.1% to achieve a chlorophyll content of 56 SPAD, by extracting 23-54 lines.

The genotypes with high chlorophyll content show a high potential of drought tolerance in accordance with the findings of other studies who stated that a more pronounced decrease of this pigment is observed in susceptible wheat genotypes [1, 15]. According to the results of other studies [4, 7] drought-tolerant wheat genotypes have higher chlorophyll content and yield compared to sensitive genotypes, indicating a positive correlation of yield and chlorophyll content under drought stress.

The usefulness of chlorophyll content as a selection criterion for drought tolerance was argued by Ahmed et al. [2] who found that the leaf chlorophyll content of tolerant genotypes was highly and positively correlated with dry weight of wheat seedlings. Also, Persic et al. [20] found that the evaluation of chlorophyll can be an effective method of selecting tolerant wheat genotypes. Drought-tolerant wheat genotypes with high chlorophyll content have also high net photosynthesis and stomatal conductance under severe drought conditions [24].

### Conclusions

For most of the crosses the inheritance of chlorophyll content was controlled by over dominance effects, associated generally with a decrease of this trait. In the case of Fundulea 4 x Turda 2000, Fundulea 4 x Alex, Fundulea 4 x GKKapos, Xenos x Turda 2000, Xenos x Apache, Fundulea 4 x Apullum and GKKapos x Apullum, the over dominance causes an increase of chlorophyll content. For Fundulea 4 x Apache and Alex x Apache the incomplete dominance leads to a decrease of chlorophyll content. For Xenos x Alex the full dominance is acting with a negative effect in the inheritance of this trait, while for Fundulea 4 x Xenos the dominance effects are very low.

The crosses Fundulea 4 x GK Kapos, Fundulea 4 x Alex and Xenos x Turda 2000 showed the highest potential to improve the chlorophyll content in wheat. In this regard, those combinations can be used as genitors or as selection material, given that there are significant variations between hybrids.

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