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# Diallel analysis of chlorophyll content in winter wheat

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**Abstract:** Chlorophyll is an essential factor in the process of photosynthesis, so that improvements of wheat yield can come about via increased photosynthesis rate. Therefore, understanding the genetic mechanism of chlorophyll content would be very important for yield improvement in wheat. The aim of this research was to assess the genetic basis of chlorophyll content, according to the gene effects and the component of genetic variance, in order to identify the best parents and crosses for future use in breeding of drought tolerance in wheat. Seven parents along with their 21 hybrids (half-diallel crosses), were studied based on diallel technique developed by Hayman and by Griffing. The expression of chlorophyll content for the studied genotypes is conditioned mainly by dominance gene action, with a low contribution of additive ones. Furthermore, the values of chlorophyll content in some parents is under the influence of genes with dominant effect, while in other parents it is under the influence of non-allelic gene interactions. The partial dominance act in the inheritance of chlorophyll content. The high non-additive effects of the cross 'Fundulea 4' x 'Apache' between parents with different (high and low) general combining ability, indicates that intermating between crosses and selection offers the possibility of obtaining genotypes with high level of chlorophyll content.

**Key words**

wheat. Inheritance, chlorophyll content, diallel.

Considering the positive correlation between chlorophyll content and photosynthetic rate [2; 19], it turns out that increasing the chlorophyll content in wheat can be an effective way to increase biomass and grain yield [21]. Leaf chlorophyll is an indicator of leaf greenness, and it is often used to analyze leaf nutrient deficiencies [1]. According to finding of Bojovic and Markovic (2009), between chlorophyll and nitrogen content of the leaf there is a very strong relation. As such, the measurement of SPAD offers the possibility to synchronize N supply with the specific demand of the crop [12]. The highly positive association between grain yield and SPAD values in wheat was found to be strong influenced by several factors, like: cultivar, environmental conditions and nitrogen content in soil [16].

Terminal drought it is considered to accelerate significantly the degradation of leaf chlorophyll [10] thus preventing carbon fixation and remobilization of assimilates [8]. The decrease of chlorophyll content under drought conditions is considered a typical symptom of oxidative stress which may be due the photo-oxidation of pigments and chlorophyll degradation [3]. Therefore, the decrease of chlorophyll content under water deficit conditions can be caused by the destruction of chloroplast membrane, an excessive swelling and deformations of vesicle blades and also the appearance of some lipid droplets [15]. A low concentration of photosynthetic pigments in leaves can cause a direct decrease of the photosynthetic potential and implicitly of the yield.

Towards a better understanding of inheritance for important traits, different statistical models and breeding strategies were developed. The genetic analysis based on diallel technique developed by Hayman (1954) and Jinks (1954), offers valuable information on the inheritance type in early generations and is useful for the achievement of effective selection. The diallel mating design was frequently used in different self and cross pollinated species in order to estimate the nature of gene action involved in the inheritance of different quantitative traits. The diallel techniques developed by Griffing (1956) regarding the analysis of combining ability, is very useful in plant breeding because it allows identifying the parents with good ability to transmit the desirable traits to offspring.

Many QTLs controlling genetics for chlorophyll content in different growth stages of wheat have been identified [17; 20], suggesting that genes involved in the inheritance of chlorophyll content represent a multilocus system [23]. Different QTLs with additive effect which simultaneously influence both grain yield and chlorophyll content were identified [22], suggesting the presence of linkage or pleiotropism. By studying the inheritance and genetic variation of chlorophyll content, it is possible to increase the efficiency and intensity of selection in wheat breeding.

The aim of this research was to assess the genetic basis of chlorophyll content, according to the gene effects and the component of genetic variance, in order to identify the best parents and crosses for future use in breeding of drought tolerance in wheat.

## Material and Method

The biologic material for this study was obtained from crosses among seven wheat varieties (‘Fundulea 4’, ‘Alex’, ‘Apullum’, ‘Turda 2000’, ‘GKKapos’, ‘Apache’, ‘Xenos’), chosen on the basis of differences in their genetic and ecologic origin. The seven parents along with their 21 hybrids (half-diallel crosses), were studied in a randomized block design with three replications.

The chlorophyll content of leaves was determined using a portable chlorophyllmeter SPAD-502 (Konica Minolta), by measuring the absorbance at 650 nm, being a non-destructive method [4]. Three readings were performed for each flag leaf, the results being expressed in SPAD units.

Data were analyzed using the method developed by Hayman (1954) for diallel crosses, relating to: analysis of genetic variance, estimation of variance (Vr) and covariance (Wr), analysis of the graph Wr-Vr. The graphical analysis was completed with the interpretation of graph based on standard deviation for parental (*yr*) and (*Wr* +*Vr*) values, according to Johnson and Aksel (1959). This second graph indicates the relationship between dominance and positive and negative alleles. The different components of genetic variance were estimated following the model of Jinks and Hayman (1953). The validity of information from diallel analysis was performed based of the significance of regression coefficient from zero and unity. The analysis of combining ability was carried out using method 2 (*p* parents and *p(p-1)/2* hybrids), model I (the model with fixed-effects, considering that the studied biological material is a population to be inferred) as suggested by Griffing (1956), using the following formula:

$$x_{ij} = u + g_i + g_j + s_{ij} + \frac{1}{bc}\sum_{k}\sum e_{ijkl}$$

where: $u$ - population mean; $g_i$ $(g_j)$ - effect of general combining ability (GCA) for parent $i$ $(j)$; $s_{ij}$ - effect of specific combining ability (SCA) for hybrid between parent $i$ and $j$; $e_{ijkl}$ - environment effect associated with individual values.

## Results and Discussions

Based on the data from Table 1, it was observed that the dominance variance (b) had the greatest and significant contribution to the chlorophyll content variability. The additive variance (a) has had a non significant value having a lower contribution to the variability of this trait than the dominance variance.

Regarding the dominance and how it acts in the inheritance of chlorophyll content, it was found that the subcomponent $b_1$of the dominance variance, which indicates the average deviation of hybrids from the parents, shoved an non significant value. As such, the effects of dominance act bidirectional; respectively in some parents it causes an increase of the chlorophyll content, while in other parents it determines the reduction of third trait.

Table 1

**Analysis of genetic variance for chlorophyll content in wheat**

| Source of variation | SS | DF | MS | F test |
|---|---|---|---|---|
| Replications | 147.57 | 2 | 737.85 | 5.56** |
| a | 111.01 | 6 | 18.50 | 1.39 |
| b | 2524.55 | 21 | 120.22 | 9.06** |
| $b_1$ | 30.56 | 1 | 30.56 | 2.30 |
| $b_2$ | 588.52 | 6 | 98.08 | 7.40** |
| $b_3$ | 1905.47 | 14 | 136.10 | 10.26** |
| Erorr | 716.18 | 54 | 13.26 | |
| Total | 3499.34 | 83 | | |

The subcomponent $b_2$ of the dominance variance by its significant value indicates an asymmetry of the distribution of positive and negative alleles that influence this trait at the parents. The significant value of subcomponent $b_3$ attests the existence of differences between the chlorophyll content of hybrids due to dominance and some dominant x dominant or additive x dominant interactions.

The regression coefficient Wr/Vr showed a significant difference from the unit, which indicates the presence of non-allelic gene interactions, such complementary or duplicative. Thus, considering the two significance tests, it seems that the values of chlorophyll content in some parents is under the influence of genes with dominant effect, while in other parents it is under the influence of non-allelic gene interactions. These two tests indicate a more complex genetic system implicated in the inheritance of chlorophyll content of these genotypes (Farooq 2015).

Table 2

**The significance of Wr/Vr regression coefficients for chlorophyll content in $F_1$ hybrids**

| Parameters | Value | Ho : β=0 | Ho : β=1 |
|---|---|---|---|
| $b$ | -0,068 | 2.49 | 39.14*** |
| $s_b$ | 0,027 | | |

Given the distance between the regression line and the parabola, as well as the position of the parents to the regression line, it follows that mainly the dominance effects play an important role in the inheritance of chlorophyll content, while the additive effects have a considerably smaller role, according to the analysis of genetic variance. Thus, based on the regression graph (Figure 1) it was observed that additive gene effects are involved in the inheritance of this trait only in the case of ‘GKKapos’ and ‘Turda 2000’ recurrent parents grouped near the regression line. Also, for other parents, the expression of chlorophyll content is under the influence of non-allelic gene interactions or environmental conditions.

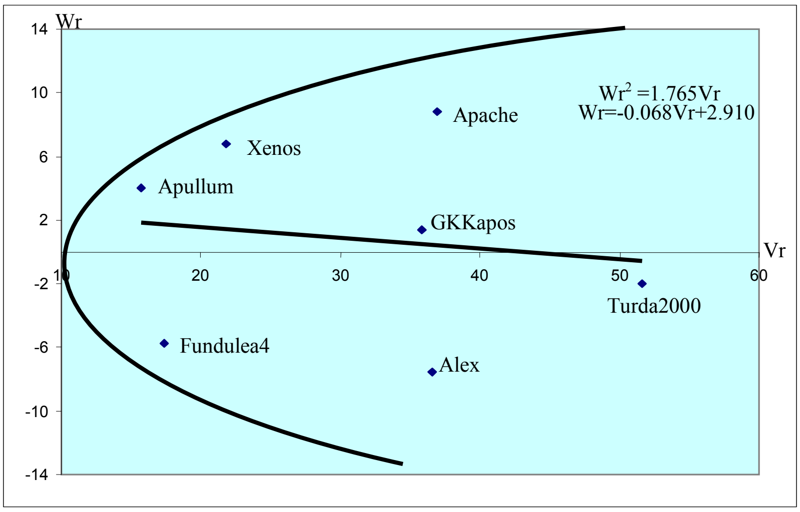

Figure 1. **Wr/Vr regression for chlorophyll content in wheat**

The regression line intersects the covariance axis above the origin (a=2.91), indicating the presence of partial dominance in the determinism of chlorophyll content, in accordance with the findings of Fellahi et al. (2017). Among the parents, 'Turda 2000' (83.80%) and 'Apache' (80.00%) varieties have the highest proportion of recessive alleles, while 'Fundulea 4' (94.40%) and 'Apullum' (67.00%) varieties have the largest proportion of dominant alleles (Table 3).

The genetic diversity between the parents for this trait can be appreciated based on their position with respect to the regression line in association with the Wr + Vr values. Thus, it was observed that the studied varieties are divided into three groups within there is a certain genetic similarity: 'Xenos'-'Apullum'-'Fundulea 4'; 'Apache' –'Alex'-'GKKapos'; and 'Turda 2000', respectively.

Table 3

**Mean (Yr), variance (Vr), covariance (Wr) values, and proportion of dominant alleles for parents concerning chlorophyll content**

| Parents | Mean Yr | Variance Vr | Covariance Wr | Vr + Wr | Proportion of dominant alleles |
|---|---|---|---|---|---|
| Fundulea 4 | 45.11 | 17.37 | -5.74 | 11.63 | 0.944 |
| Xenos | 49.18 | 21.78 | 6.78 | 28.56 | 0.496 |
| GKKapos | 47.23 | 35.80 | 1.39 | 37.19 | 0.420 |
| Turda 2000 | 46.38 | 51.58 | -2.02 | 49.56 | 0.162 |
| Alex | 43.00 | 36.53 | -7.54 | 28.99 | 0.641 |
| Apache | 50.25 | 36.96 | 8.79 | 45.75 | 0.200 |
| Apullum | 48.32 | 15.73 | 4.04 | 19.77 | 0.670 |

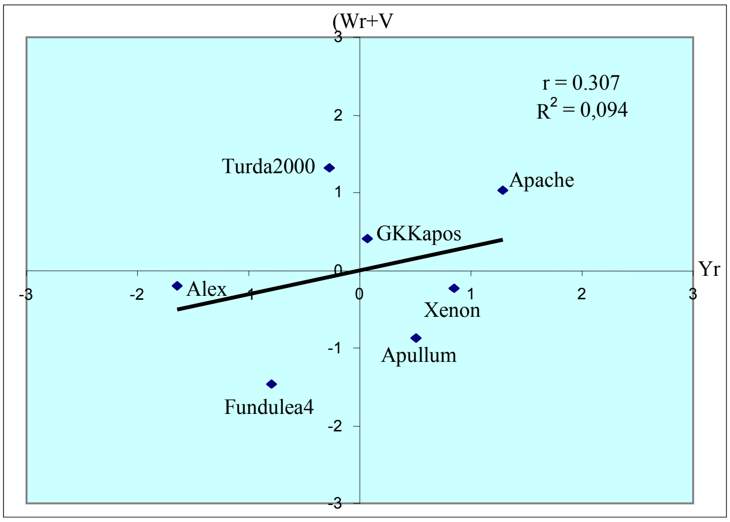

Figure 2. **Standardized deviations (Wr+Vr) and parents mean (yr) for chlorophyll content in wheat**

The distribution of the parents based on the standardized deviations yr (chlorophyll content) and (Wr+Vr), indicates a high proportion of recessive alleles trait in 'Apache' and 'GKKapos' varieties, while in 'Turda 2000' variety the recessive alleles cause a reduction of the chlorophyll content.

In the case of 'Fundulea 4' and 'Alex' varieties, the dominance was associated with negative alleles, while in 'Apullum' and 'Xenos' varieties the dominance was associated with alleles that increase the chlorophyll content.

The dominance effects ($H_1$) play a major and very significant contribution in genetic determinism of this trait (Table 4), while the additive effects (D) have a small and insignificant contribution, in accordance with the analysis of genetic variance and with the results of Fellahi et al. (2017).

Table 4

**Components of variance and genetic parameters for chlorophyll content in wheat**

| Variance component / ratio | Estimated values |
|---|---|
| *D*- additive effects of genes | 1.765 |
| $H_1$- dominance effects of genes | 121.804*** |
| $H_2$ – corrected dominance effects of genes | 95.101*** |
| *F*- covariance of additive and dominance effects | 0.276 |
| $h^2$- cumulative dominance effects | 3.701* |
| *E*- environmental variance | 4.401* |
| $(H_1/D)^{1/2}$ – average level of dominance | 8.306 |
| *kD/(kD+kR)* – proportion of dominance genes | 0.505 |
| $\overline{F_1} - \overline{P}$ - average direction of dominance | -1.40 |
| $D-H_1$ – average direction of genes effects | -120.04 |
| $H_2/4H_1$ – average frequency of positive and negative alleles | 0.195 |
| $h^2/H_2$ – number of blocks of dominant genes | 0.045 |
| D/(D+E) – true sense heritability | 0.287 |
| *Hb* – broad sense heritability | 0.896 |
| *Hn* – narrow sense heritability | 0.333 |

$LSD_{5\%} = 3.518$ $LSD_{1\%} = 4.751$ $LSD_{0.1\%} = 6.327$

The predominant value of the dominance effects was supported by the significant value of the $h^2$ component, which represents the sum of all heterozygous loci. The dominant alleles that control the chlorophyll content (F) have a frequency (50.50%) close to the recessive ones (49.50%) and at the same time there is an asymmetry of the positive and negative effects of the genes due to dominance, considering the significant value of $H_2$ component.

High values of broad sense heritability (0.896%) as well as low values of narrow sense heritability (0.333) confirms that a considerable part of the chlorophyll content variability is due to genotype, amid the involvement of some major genes in the inheritance of this trait. Perez et al. (2020), reported high heritability of SPAD in wheat suggesting the importance of this trait for yield improvement. Low value of narrow sense heritability for chlorophyll content was also found by Fellahi et al. (2017).

The environment variance (E) even if it has a significant value, is lower than the components related to the effects of dominance (H1) and additivity (D). Based on the H2/4H1 ratios (0.195≠0.250) it was observed that the dominant genes are in different frequencies positive and negative having positive effects on the chlorophyll content in some parents and negative effects in others.

Based on the data from Table 5, it was observed that in the chlorophyll content variability at F1 hybrids, both the additive and non-additive gene effects have a major and significant influence. The values of the GCA/SCA ratio indicate a predominant and significantly superior action of the additive effects of approximately 70% in the inheritance of this trait.

Table 5

**Analysis of variance for combining ability of chlorophyll content in wheat**

| Variability source | SS | DF | MS | F |
|---|---|---|---|---|
| Total | 3170.36 | 62 | | |
| Replication | 125.86 | 2 | 62.93 | 4.57* |
| GCA | 1256.13 | 6 | 209.35 | 15.21** |
| SCA | 1237.88 | 14 | 88.42 | 6.43** |
| Error | 550.47 | 40 | 13.76 | |

GCA/SCA 2.36**

The effects of the general combining ability (GCA) present a higher proportion of negative values, with amplitude of 11.64, from -5.06 at 'Alex' to 6.58 at 'Fundulea 4' variety. Thus, 'Fundulea 4' variety which possesses a high potential for improving drought resistance according to this trait, has the ability to transmit in the offspring high chlorophyll content. 'Alex' (-5.06) and 'Apache' (-2.86) varieties recorded the lowest additive effects to improve this trait. The average effects of the general combining ability are very weak correlated with the values of this trait in the parents (r=0.084).

Table 6

**Effects of the general and specific combining ability for chlorophyll content in wheat**

| Parents | GCA | SCA | | | | | |
|---|---|---|---|---|---|---|---|
| | | Xenos | GKKapos | Turda 2000 | Alex | Apache | Apullum |
| Fundulea 4 | 6.58* | | -7.54 | 3.81 | 1.31 | 7.73 | -4.10 |
| Xenos | 2.21 | | | -2.11 | 7.99 | -3.26 | 6.89 |
| GKKapos | -0.53 | | | | -0.08 | -4.23 | -0.85 |
| Turda 2000 | -0.99 | | | | | -0.41 | -8.35 |
| Alex | -5.06 | | | | | | 3.24 |
| Apache | -2.86 | | | | | | |
| Apullum | 0.67 | | | | | | |

GCA -LSD$_{5\%}$ =6.1357; LSD$_{1\%}$ =8.18; LSD$_{0.1\%}$ =10.69;
SCA -LSD$_{5\%}$ = 8.91; LSD$_{1\%}$ =11.89; LSD$_{0.1\%}$ =15.54

The effects of the specific combining ability (SCA) presented different values, mostly negative and non significant with a range of 16.34, from -8.35 for ‘Turda 2000’ x ‘Apache’ to 7.99 for ‘Xenos’ x ‘Turda 2000’ hybrid. The highest non-additive effects in this generation were observed at the combinations: ‘Xenos’ x ‘Turda 2000’ (7.99), ‘Fundulea 4’ x ‘Alex’ (7.73) and ‘Xenos x Apache’ (6.89), which offers the possibility of obtaining valuable genotypes for chlorophyll content and drought tolerance in segregating generations.

## Conclusions

The expression of chlorophyll content for the studied genotypes is conditioned mainly by dominance gene action, with a low contribution of additive ones. Furthermore, the values of chlorophyll content in some parents is under the influence of genes with dominant effect, while in other parents it is under the influence of non-allelic gene interactions. The partial dominance act in the inheritance of chlorophyll content. Considering the genetic diversity between the parents, it turns out that they possess different gene combinations that control this trait, thus offering the possibility of an efficient choice of parents for hybridization.

Given the high values of two types of heritability it seems that a considerable part of the chlorophyll content variability is due to genotype, amid the involvement of some major genes in the inheritance of this trait. As such, the delay of selection can be an effective way for the improvement of this trait. The high non-additive effects of the cross ‘Fundulea 4’ x ‘Apache’ between parents with different (high and low) GCA, indicates that intermating between crosses and selection offers the possibility of obtaining genotypes with high level of chlorophyll content.

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