Morphological observations in durum wheat after embryo manipulations

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Abstract: In cereals, haploid or diploid embryo structures are suitable organisations in practice for amelioration or storage of genotypes or some other biotechnologies. It is possible to reveal of variation as different genotypes from heterozygote or landraces after making homozygote of recessive alleles by doubling of chromosomes using embryo rescue. In these genotypes, characters can be evaluated as qualitative or quantitatively, for instance; dark purple coleoptile could be observed in some doubled haploid (DH) genotypes. The amount of chlorophyll (a/b) and carotenoid have been evaluated in young leaves in DH-6 and DH-8 and some other durum wheat genotypes, while investigating their tolerances to salinity in laboratory conditions. Colour of grains (amount of carotenoid in harvested grains) in durum genotypes have also been evaluated with field experiment conditions in three different locations of Anatolia and also correlation (r) has been identified with other grain characters related with agronomical values. Here it is possible to determine the rate into correlation (path) of characters and direct and indirect effects of each other. Naturally, the relationships between amount of chlorophyll in young leaves and carotenoid in harvested grains and also harvest index (HI) of genotypes will be able to be interesting subjects to discuss.

1. Introduction
Morphogenesis is the shaping of an organism by embryiological processes of differentiation of cells, tissues and organs and the development of organ systems according to the genetic “blueprint” of the potential organism and environmental conditions [1]. Possible mechanism by which the genes of zygote may determine anatomical structure had been explained by Turing (1952) with chemistry and mathematic model of growing embryo [1]. In cereals, morphological and anatomical structure of embryo and embryological processes of differentiation in either haploid or diploid structure and also their exploitation have been explained by Savaskan [2]. Now before zygote in cereals, it can be intervened to meiosis in an egg cell using wide crosses to produce haploid embryo [3] and be changed the morphology of an individual plant by doubling of chromosomes from a haploid plantlet. Doubled haploid (DH) procedure can be realized with some other advanced techniques, for instance microspore culture [4].

Actually, the importance of exploitation of genetic resources (landraces, wild species, obsolete cultivars) using advanced genetic techniques (wide crosses etc.) in breeding programs had been explained detail by Porceddu et al in 1988 [5]. It is possible to reveal of variation as different genotypes after making homozygote of dominant (‘AA’) and recessive (‘aa’) alleles by doubling of chromosomes using embryo rescue, from heterozygote or landraces [6]. In these genotypes, characters can be evaluated as qualitative or quantitatively in genetics and agronomy for instance, dark purple coleoptile could be observed in some of DH genotypes (Figure 1a). In wheat, recessive allele (‘a’) of ‘Rc’ gene located on
the short arm of 7 number on homeolog chromosome produce dark coleoptile color in germination and it is close to resistant gene to *Puccinia triticina* [7].

Besides this, two different DH genotypes have been investigated with progenitor *Triticum durum* cv. Kunduru 1149 and some other durum wheat cultivars for salinity tolerance measuring chlorophyll a, b and carotenoid amounts in young leaves in laboratory conditions [8]. Ameliorated durum genotypes DH-6 and DH-8 showed higher amount of total chlorophyll, chlorophyll a and b and also carotenoid than an Anatolian landrace Kunduru 1149 and other durum cultivars (table 1). On the other hand, colour in harvested grains of DH-6 and DH-8 have also been identified after field experiments and found different amount of carotenoid 6.25 and 6.58 ppm respectively (Figure 1b).

![Figure 1](image)

*Figure 1.* Coleoptiles, dark purple in DH-8, 9 and green in 10 (left) and carotenoid in harvested grains DH-8 (6.58 ppm) and 6 (6.25 ppm) genotypes (right).

**Table 1.** Chlorophyll a, b and carotenoid amounts in leaf tissue (g) of 35 day of plants in durum wheat genotypes

| Genotypes      | Total chlorophyll mg g⁻¹ plant | Chlorophyll a mg g⁻¹ plant | Chlorophyll b mg g⁻¹ plant | Chlorophyll a/b mg g⁻¹ plant | Carotenoid |
|----------------|--------------------------------|---------------------------|----------------------------|----------------------------|------------|
| Mirzabey       | 7.52c*                         | 5.72c                     | 1.80c                      | 3.17                       | 1.070b     |
| Altın          | 5.79d                          | 3.99d                     | 1.80c                      | 2.21                       | 0.946b     |
| Kunduru 1149   | 12.28b                         | 7.39b                     | 4.88b                      | 1.51                       | 1.115b     |
| DH-6           | 19.79a                         | 12.40a                    | 7.43a                      | 1.66                       | 1.766a     |
| DH-8           | 19.48a                         | 12.32a                    | 7.10a                      | 1.73                       | 1.330b     |

*Entries within column followed by same letter are not significantly different (0.05).

This table summarised from the tables which are found in Duran et al, 2010 [8].

Some of grain characters related with agronomical importance of DH genotypes have been evaluated after harvesting either in one or in three different location of Anatolia identifying their morphology with classical measurements and statistics [9]. Simple correlation coefficient (r) is very useful statistics to understand the relationships between two independent variables. However, the degree of interaction may be need other statistics to make explanation the details of relationships.

The subject of this presentation is:

1. to understand the share of direct and indirect effects between characters into the correlation using path analysis, on the other hand,
2. to discuss if it might be possible the relationships between the amount of chlorophyll in young leaves and carotenoid in harvested grains and also harvest index (HI) of genotypes.

### 2. Materials and methods

#### 2.1. Plant materials
In our researches, mainly durum wheat has been used as plant material. Wheat is one of the most important crop plants economically and has a long period of evolutionary history. Twenty two individual DH durum wheat genotypes and their three progenitors (\textit{T.durum} Desf. cvs. Kunduru 1149, Berkmen 469 and Cakmak 79) were used for this investigation [9].

2.2. Field experiments and statistical analysis
Field experiment: Plants of each genotype and parents were grown in five row plots in each of three randomized blocks from a winter sowing in Ankara. Each block contained totally 22 DH genotypes and 3 parents. Rows were spaced 20 cm apart and 20 seeds spaced 5 cm apart within each row. From each replication of genotypes, 12 seeds of length, width and thickness were measured with a compass mounted on a stereomicroscope (Olympus X-Tr). An estimate of seed volume was obtained by multiplying the seed length x width x thickness (mm$^3$) and the ratio of individual seed weight/volume (mg/mm$^3$) provided an estimate of seed density [10]. Grain weight (mg) was found by dividing to 1000 of the 1000-grain weight of DH genotypes in each replication. Micro-Kjeldahl procedure was used to determine total N values of genotypes and multiplied by the factor 6.25 for protein amount. The butanol test was used to determine pigment value (ppm) of carotenoid. Absorbance value of carotenoid was evaluated in an spectrometer (Unicam UV4-200, $\lambda$ = 435.8 nm).

Statistical analysis can be described as Savaskan et al 2003 [9]. For path analysis, all data belonging to replications were performed by MSTAT-C statistical package program after analysis of variance and simple correlation coefficient (r) calculations [10]. Means of genotypes were compared with Least Significant Differences (LSD) test [11].

3. Results and discussion
3.1. Path analysis according to the results of field experiments
Analysis of variance were performed according to the results of basic grain characters in one location (table 2). They showed differences in DH genotypes derived from Kunduru 1149 in two probability levels (P<0.01) (P<0.05) (table 2). Volume was correlated (r) significantly with all characters shown in table 3. It was correlated important positive with color (carotenoid as ppm), weight and vitreousness and negative with density and total N (protein) significantly (P<0.01) (table 3).

Volume and density correlated negative (table 3 and 4) and direct effect of density on volume (path) was found as -0.5349 and percentage into the correlation coefficient as 71.16% (table 4). Direct effect of total N (protein) on volume was found as 0.0103 and percentage into the correlation coefficient was very low (1.7199%) (table 4) however indirect negative effects of density and weight (-36.10 and -58.39% subsequently) with protein on volume increased the simple correlation coefficient (r = -0.576**) (table 4 and 3).

Table 2. The summarize of variance analysis of grain characters in DH genotypes and their progenitors.

| Grain characters     | DH genotypes derived from \textit{T.durum} Desf. cvs. |
|----------------------|-----------------------------------------------|
|                      | Kunduru 1149 | Berkmen 469 | Cakmak 79 |
| Grain weight (mg)    | 30.854**     | 0.597n.s.   | 2.025n.s. |
| Total N              | 5.971**      | 0.572n.s.   | 0.112n.s. |
| Carotenoid (ppm)     | 1852.043**   | 774.894**   | 665.970** |
| Estimated volume (mm3)| 16.765**     | 10.671**    | 0.724n.s. |
| Est. density (mg/mm3)| 2.425*       | 2.181n.s.   | 0.118n.s. |
| Length (mm)          | 20.958**     | 15.911*     | 2.623n.s. |
| Error s.d.           | 14           | 5           | 3         |

**Significant at the 0.01, * significant at the 0.05 probability levels, ns=no significant [9]
Table 3. Simple correlation coefficients (r) between the grain characters for DH genotypes derived from T. durum cv. Kunduru 1149.

| Genotypes | Density | Total N | Weight | Colour | Vitreousness |
|-----------|---------|---------|--------|--------|--------------|
| Volume    | -0.774**| -0.576**| 0.827**| 0.569**| 0.469**      |
| Density   | 1       | 0.402**| -0.324*| -0.180n.s.| -0.294*      |
| Total N   | 1       | -0.588**| -0.207n.s.| -0.217n.s.|             |
| Weight    | 1       | 0.628**| 0.486**|        |              |
| Colour    |         |         |        | 0.643**|              |
| Vitreousness |       |         |        |        |              |

**Significant at the 0.01, * significant at the 0.05 probability levels, ns=no significant [9]

Direct effect of weight on volume was found as 0.5918 and the share into correlation coefficient was 71.54% (table 4). The share of weight was found 25.50% percentage into correlation coefficient indirectly (Table 4). Direct effect of protein on volume was found as positive (0.0103) and its percentage into correlation coefficient (r) was also found as very low value (1.7199%). However indirect negative effects of density and weight very high values across to direct effect of protein value on volume (36.10 and 58.39% respectively) (table 4) and those two values must be supportive factors between the negative important relationships (r) of volume and protein (P<0.01) (table 3).

There is not found any correlation between color and protein (total N) as it can be seen in table 3 (-0.207n.s.). Because the locations and missions of protein and carotenoid can be interpreted as different in a seed structure. Color positive correlated with either volume or weight (table 3). Carotenoid covers all of the harvested biomass and localized mainly into seed coat in seed (grain). It may be in relationships slightly less amount with endosperm (starch) and embryo (protein etc.). Direct effect of weight on volume was found as 0.59.18 and found very big percentage into correlation coefficient, 70.54% (Table 4). The indirect effects of density and color on volume with weight were found as 0.173 and -0.060 subsequently and also their share was found as 20.66% and 7.49% (table 4).

Table 4. Direct and indirect effects of density, weight, total N, colour and grain length on volume in all DH genotypes derived from Kunduru 1149.

| Direct and indirect effects | PATH coefficient | Percentage into the correlation coefficient (r) |
|-----------------------------|------------------|-----------------------------------------------|
| **Direct effect**           |                  |                                               |
| Density:                    | -0.5349          | 71.16%                                        |
| **Indirect effects**        |                  |                                               |
| Weight:                     | -0.1917          | 25.50%                                        |
| Other characters: (colour, protein, length) | -0.0251 | 3.34%                                        |
| **Direct effect**           |                  |                                               |
| Protein:                    | 0.0103           | 1.719%                                        |
| **Indirect effects**        |                  |                                               |
| Density:                    | -0.2152          | 36.10%                                        |
| Weight:                     | -0.3480          | 58.39%                                        |
| Other characters: (colour, length) | -0.0227 | 3.80%                                        |
| **Direct effect**           |                  |                                               |
| Weight:                     | 0.5918           | 70.54%                                        |
| **Indirect effect**         |                  |                                               |
| Density:                    | 0.173            | 20.66%                                        |
| Colour:                     | -0.060           | 7.49%                                         |
| Other characters: (protein, length) | 0.0109 | 1.30%                                        |
3.2. Relationships between chlorophyll in young leaves and carotenoid in harvested grains and also harvest index (HI):

According to molecular analysis of DH durum lines derived from an Anatolian landrace Kunduru 1149, DH-1, 11, 4, 6 and 7 have been determined as identical [6]. On the other hand, DH-2 and 9 and also DH-5 and 8 were identified as similar individuals. [6]. As a result of this molecular identification DH-6 and 8 are different homozygote genotypes and they showed resistance to salinity differently in a plant growth chamber conditions, in 200 mM NaCl concentration and under 12 klux/m² and 16/8 day/night application [8]. High amount of chlorophyll in young leaves must be important as it will be biomass value and it is a strong indicator for high photosynthesis capacity in a plant. Ameliorated genotypes DH-6 and 8 produced higher chlorophyll amount (total, a and b) than Kunduru 1149 (progenitor) and other durum wheat cultivars significantly (P<0.05) (table 1) [8]. HI of DH-6 and 8 were calculated as 43.93 and 36.58 respectively (table 5). There are ten of qualitative and quantitative characters belonging to DH genotypes derived from Kunduru 1149 in the results of experiments after winter cultivation (table 5). DH-6 and 8 have different amount of carotenoid (color) in harvested grains (6.25 and 6.58 ppm respectively) (Figure 1, right) (table 5).

Table 5. Means and LSD test of qualitative and quantitative characters belonging to DH genotypes derived from Kunduru 1149 after winter cultivation.

| DH | Headline | Plant Height (cm) | Spike length (cm) | Spikelet on Spike (n) | Grain on spike (n) | Grain weight (1000 grain) (g) | Yield/plant (g) | Tiller HI (n) | Protein amount (%) | Caroten in grain (ppm) |
|----|----------|------------------|------------------|----------------------|--------------------|-----------------------------|----------------|---------------|-----------------|---------------------|
| 6  | 11.00ab  | 134.53a          | 8.33ab           | 21.18abc             | 52.09ab            | 57.98ab                     | 16.13abc       | 7.49bc        | 14.13def         | 6.25               |
| 7  | 11.67abc | 133.26ab         | 8.27bcd          | 21.06ab              | 55.41a             | 54.26cde                    | 17.28ab        | 7.60bcd       | 14.02cd           | 6.16c               |
| 8  | 16.00cd  | 131.71ab         | 8.33bdf          | 21.17abc             | 53.13ab            | 52.14ef                     | 16.24abc       | 8.67b         | 15.37ab           | 6.58b               |
| 9  | 17.34e   | 126.64bc         | 7.73ef           | 19.60bc              | 49.80bc            | 47.60ef                     | 11.74bcd       | 6.62bcd       | 15.71e            | 6.46d               |
| 10 | 14.00cd  | 128.91ab         | 7.71f            | 19.94abc             | 50.17ab            | 53.58eef                    | 15.74abc       | 8.12bc        | 14.41bc           | 5.88c               |

**Significant at the 0.01, * significant at the 0.05 probability levels, ns=no significant + days after 15th May; Entries within column followed by same letter are not significantly different (0.05). HI = grain weight / biomass X 100, biomass = grain weight + dry matter

4. Conclusion

1. Morphogenesis is a complicative chain of systems which begins from zygote by taking of its genetics potential and also environmental conditions and other disciplines [1]. In cereals an intervention to the meiosis with advanced technologies [2,3,4] can change the capacity of embryo genetically. Landraces or local populations are important resources for genetic practises and in agronomy for breeding methods in wheat [5]. Ameliorated durum wheat genotypes will give more promising morphology in cultivation than their progenitors (landraces, old/obselet cultivars).

2. Path analysis were performed between grain characters and found direct and indirect effects of share inside in simple correlation, coefficient (r) (table 4). That statistics can be used for detail in relationships between two independent parameters.

3. High amount of chlorophyll in young leaves must be important as it will be biomass value. It is a strong indicator for high photosynthesis capacity in a plant. In morphology such as tillering, plant height, spike length, grain on spike or grain weight (table 5) are effective characters on biomass and dry matter after harvesting. HI values of durum wheat DH genotypes derived from Kunduru 1149 can be seen in table 5.

4. Some of recessive characters cannot be seen in population until homozygosity such as, dark purple coleoptile (DH-8 and 9) (Figure 1 left). Also length of spikelet stem (DH-3) or position (array) of grains on spike stem (DH-2 with angle and DH-3 with smooth, derived from T. durum Desf.
Berkmen 469) are observed after homozygosity. However, these kinds of features can be considered as enrichments of genotypes basically and open to interests in breeding programmes.

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