

# The genetic control of the alpha-amylase isozymes of the durum wheat (*Triticum durum* Desf.)

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The electrophoretic spectrum of alpha-amylase isozymes of both common (*T.aestivum* L.) and durum (*T.durum* Desf.) wheat contains in the malt-zone invariable triplet of the components. The genetic control of this triplet is yet undetermined due to its immutability among any Chinese Spring nulli-tetrasomics for 6th and 7th homologous groups. The question about localization of genes that control permanent bands of the malt-zone is quite important while these genes are suitable and efficient chromosome-specific markers, which demonstrate moderate polymorphism among common and durum wheat genotypes. The objective of this investigation was to ascertain the genetic control durum wheat of  $\alpha$ -amylase isozymes that form the invariable malt-zone triplet.

## Genetic stocks used in the research:

a) forms and cultivars with the genomic formula AABB,  $2n=28$ , namely durum winter wheat cultivar **Chornomor** (developer – Palamarchuk O. I., Odesa), durum winter lines **Leucurum**, **Rubrum**, and **Candicance**, kindly provided by Plant Growing Institute after Jurjiv V. Ja., Kharkiv, winter durum wheat line **Mutiko italicum 59h132**, developed and kindly provided by breeder Kostin V. V., KNIAC, Krasnodar. The mentioned parental forms were crossed after the diallelic scheme without reciprocal crossings. The  $F_2$  plants were allowed to self-pollination under the isolators. The  $F_2$  individuals were grown, and seeds obtained from each plant were used in this investigation for  $\alpha$ -amylase extraction.

b) for  $\alpha$ -amylase spectra obtainment following stocks were also used: common wheat cultivars Chinese Spring, Kavkaz, *Avrora* (the genomic formula AABBDD), and species *T. boeoticum* Boiss. ( $A^a A^a$ ), *T. timopheevii* Zhuk. ( $ABAbGG$ ), a nulli-tetrasomics for 6th and 7th homologous groups, developed from Chinese Spring cultivar, in all possible combinations.

The polymorphism in the invariable triplet was observed among five durum wheat genotypes (Fig. 1, a–e). The spectrum of Leucurum lacks band 4 (Fig. 1c), and demonstrates single bands 5 and 6, whereas the spectrum of MI (Fig. 1d) has band 4, and shows double bands 5 and 6. The invariable triplet spectra of Rubrum and Candicance have all three components (Fig. 1a, e), and Chornomor's spectrum (Fig. 1b) has bands 4, 5, and double 6. The availability of the polymorphism in the malt-zone triplet among some durum wheat representatives provides a possibility of using these genetic stocks in the genetic analysis of the triplet's components genetic control.

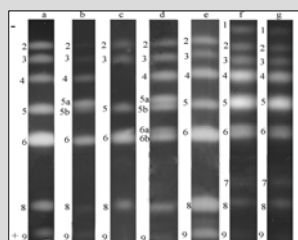


Fig. 1. The electrophoretic spectra of some durum and common wheat genotypes. a – Rubrum, b – Chornomor, c – Leucurum, d – Mutiko italicum, e – Candicance, f – Avrora, g – Kavkaz.

The electrophoretic spectra of different nulli-tetrasomics for the 6th homologous group approve previous results and provide evidence that whichever chromosome was absent, none of the triplet's bands disappeared. So, we may presume that isozymes, which genes locate on different chromosomes, may have the same electrophoretic mobility and therefore collocate on the spectrum. To check this assumption, we examined the spectra of the diploid wheat *T. boeoticum* Boiss. ( $A^a A^a$  genome) and tetraploid wheat *T. timopheevii* Zhuk. ( $A^a A^a GG$  genome).

The spectrum of diploid species *T. boeoticum* is presented only by two bands, which are both located in the invariable triplet zone. These bands are the 5th and the 6th, and the latter could vary among different einkorn samples and be either single or double. (Fig. 2a, b).

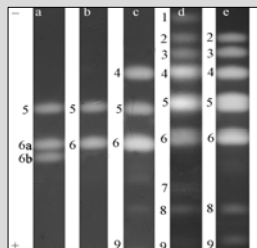


Fig. 2. The electrophoretic spectra of  $\alpha$ -amylase isozymes of some *Triticum* species genotypes. a, b – *T. boeoticum*, c, d – *T. timopheevii*, e – durum wheat cultivar Avrora, e – durum wheat cultivar Chornomor.

Wild einkorn, the boeotic wheat, also lacks on its spectrum bands 3 and 7, which are controlled by chromosomes of common wheat's  $A^a$  subgenome. Therefore, we could presume that the initial gene of the einkorn wheat of the  $A^a$  subgenome is duplicated in comparison to genome  $A^a$ , and these genes encode different bands. So, the comparison of spectra of diploid, two tetraploid and one hexaploid wheat provides evidence that the upper band of the triplet is encoded by G (B) subgenome, but leaves unclear how the next two bands are controlled. The other assumption occurs: if two bands of triplet, 5 and 6, are controlled by one genome ( $A^a$  of *T. boeoticum*), it might be possible that B or G subgenomes, as well as D one of common wheat, may encode two isozymes each. If some isozymes have equal electrophoretic mobility, they should collocate on the spectrum and as a result decrease the number of the bands on spectrum and form the invariable triplet. Moreover, that should cause ineffectiveness of nulli-tetrasomics exploitation in gene location definition. Figure 3 demonstrates two most plain schemes of electrophoretic bands' genetic control in hexaploid wheats. It should be noted that neither of these schemes for common wheat, a and b, is more trustworthy than the other due to absence of the appropriate genetic stocks.

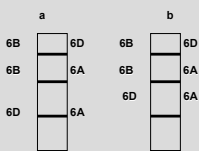


Fig. 3. Some variants of chromosomal control of bands in invariable triplet of  $\alpha$ -amylase in common wheat

One of the  $\alpha$ -amylase bands of the *T. boeoticum* is controlled by *Amy-A1* (6A), and the other is controlled by *Amy-A3* (5A). Figure 4 demonstrates six most plain schemes of electrophoretic bands' genetic control in tetraploid wheats, which explain the presence of three components in invariable spectrum part.

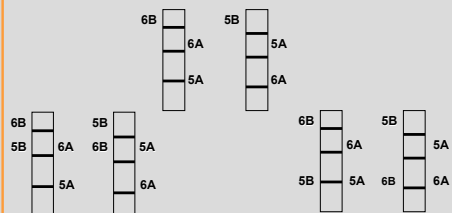


Fig. 4. Some variants of chromosomal control of bands in invariable triplet of  $\alpha$ -amylase in durum wheat

For the genetic analysis combinations Leucurum x Rubrum, MI x Leucurum, and MI x Rubrum were used due to their difference in the  $\alpha$ -amylase malt-zone triplet on the electrophoretic spectra (Fig. 1). Four seeds from each  $F_2$  individual were randomly taken, and the extracted  $\alpha$ -amylase was separated electrophoretically. On the basis of the four seeds spectra the genotype for genes that control  $\alpha$ -amylase of each  $F_2$  individual was reconstructed.

As cultivar Leucurum has no upper band on its spectrum, we can not distinguish a homozygote for its alternative allele, which demonstrates a band, from a heterozygote for this gene. So, there could be only two phenotypic classes: homozygotes for the nulli-allele (no band) and general class that consists of homo- and heterozygotes, demonstrating the upper band. The same concerns the second and the third bands of triplet. The double bands provided by MI genotype collide with the respective single bands of other genotypes; therefore, according to genes that control two lower triplet bands  $F_2$  plants could be divided into two phenotypic classes: homozygotes with the single band phenotype, and homo- and heterozygotes with the double bands phenotype.

Figure 5 and 6 demonstrate electrophoretic spectra of  $F_3$  individuals obtained from the crossing between genotypes MI and Leucurum (Fig. 5), Leucurum and Rubrum (Fig. 6), bands are defined via letter (that means corresponding cultivar) and number (that means the position in the triplet zone). Bands M1 and R1, R2 and L2, R3 and L3 are not analyzed due to their identity. Bands M1 to R1 dominate over L1; consequently, M1M1 and M1L1, R1R1 and R1L1 are identical, too. In the same way, band M2 dominates over R2, and M3 – R3, and genotypes M2M2 and M2R2, M3M3 and M3R3 could not be distinguished.

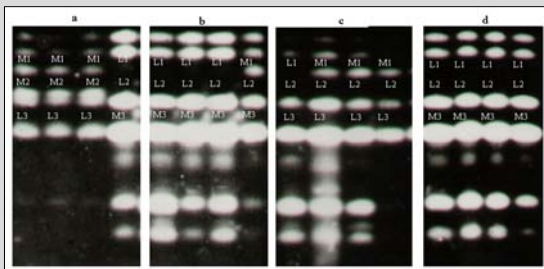


Fig. 5. Some examples of  $\alpha$ -amylase electrophoretic spectra of four  $F_3$  seed grown on the  $F_2$  plant, crossing combination MI x Leucurum. a – M1L1M2L2M3L3 genotype of the parent plant  $F_2$ , b – M1L1L2L2M3M3, c – M1L1L2L2L3L3, d – L1L1L2L2M3M3.

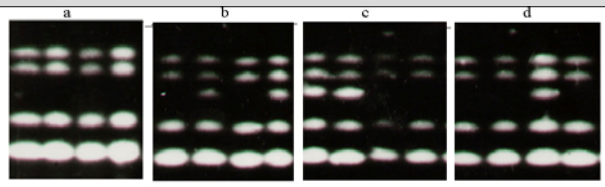


Fig. 6. Some examples of  $\alpha$ -amylase electrophoretic spectra of four  $F_3$  seed grown on the  $F_2$  plant, crossing combination Leucurum x Rubrum. a – L1L1 genotype of the parent plant  $F_2$ , b, c, d – L1R1.

The distribution of the  $F_2$  plants into phenotypic classes (Table 1) agrees with the theoretically expected one in those two crossing combinations, where Leucurum was one of the parental forms. The empiric distribution differs from the theoretic one in the combination, in which MI was one of the parental forms. A deficit of plants in classes with bands M2 and M3 is observed. MI has rather weak frost-resistance, so, it is fear to presume that among  $F_2$  individuals did not winter those ones that possessed, first of all, originated from MI 5A chromosome, which is known as a carrier of several genes that control frost resistance. Under negative selection occurred genes located on the 5A chromosome, among which was *Amy-A3* gene. Consequently, we could presume that the central band of the invariable malt-zone triplet is controlled exactly by this gene. So, schemes "d" and "f" in Fig. 4 can be thought of as more realistic as for chromosomal control of the bands in the invariable part of  $\alpha$ -amylase spectrum of durum wheat.

Table  
The distribution of  $F_2$  individuals into phenotypic classes after  $\alpha$ -amylase electrophoretic spectrum bands in the analyzed combinations

| Phenotypic classes | Empiric classes' volumes | Theoretic classes' volumes | Phenotypic classes | Empiric classes' volumes | Theoretic classes' volumes |
|--------------------|--------------------------|----------------------------|--------------------|--------------------------|----------------------------|
| Leucurum x MI      |                          |                            | Leucurum x Rubrum  |                          |                            |
| 27 M1M2M3          | 65                       | 60,75                      | 3 R1               | 76                       | 74,25                      |
| 9 M1M2L3           | 19                       | 20,25                      | 1 L1               | 23                       | 24,25                      |
| 9 M1L2M3           | 18                       | 20,25                      | Total              | 99                       |                            |
| 9 L1M2M3           | 17                       | 20,25                      | $\chi^2$ value     |                          | 0,16 < $\chi^2_{0,01}$     |
| 3 M1L2L3           | 8                        | 6,75                       | MI x Rubrum        |                          |                            |
| 3 L1L2M3           | 2                        | 6,75                       | 9 M2M3             | 48                       | 63                         |
| 3 L1M2L3           | 12                       | 6,75                       | 3 M2R3             | 21                       | 21                         |
| 1 L1L2L3           | 3                        | 2,25                       | 3 R2M3             | 31                       | 21                         |
| Total              | 144                      |                            | 1 R2R3             | 12                       | 7                          |
| $\chi^2$ value     |                          | 9,05 < $\chi^2_{0,01}$     |                    | 112                      | 20,96 > $\chi^2_{0,01}$    |

## Conclusion

The invariable malt-zone triplet of  $\alpha$ -amylase in tetraploid wheats with genome formulas  $A^a A^a BB$  and  $A^a A^a GG$  is controlled by three genes, which recombine independently.

Two of them are controlled by genes of the A subgenome: the lowest band is a product of the *Amy-A1*, located on the 6A chromosome, the central triplet's band is a product of *Amy-A3*, located on 5A chromosome.

The upper band is encoded by subgenomes B of *T. durum* and G of *T. timopheevii*.