

VARIABILITY OF NUCLEIC ACIDS CONTENT IN THE LEAVES OF *TĂMÂIOASA ROMÂNEASCĂ* GRAPEVINE VARIETY

Silvica PĂDUREANU¹

¹University of Agronomy Sciences and Veterinary
Medicine of Iasi, Faculty of Agriculture, Cell
Biology Department, 3,
M. Sadoveanu Alley, 700490, Iassy, Romania
e-mail: silvyp27@yahoo.com

The paper presents the variability of the DNA and RNA content of a mature leaf belonging to the Tămâioasă românească grapevine cultivar. The analyse has been effectuated in four phenological phases: unbudding, flowering, ripening, full maturation of grapes. On constate that at unbudding, the nucleic acids content is maximum, because in this phases, the proteinic synthesis are very intense for cell organits biogenesis. În other phases, the content in DNA and RNA diminish very much. Therefore, the content of DNA and RNA is maximum at unbudding and minimum at full maturation of grapes. The content of DNA in vegetal cell is relative constant, between certain limits, while the content of RNA is enough variable. During the vegetation period, DNA content in the mature leaf of Tămâioasă românească grapevine variety is much higher compared to RNA content. The variability of DNA content is more restricted compared to the one of RNA content. RNA/DNA ratio from mature leaf of Tămâioasă românească grapevine variety is maximum at the phenological phase of full maturation of grapes and minimum at the phenological phases of flowering. The estimate of proportion between of contents in RNA and DNA in mature leaves at Tămâioasă românească grapevine variety permit certain observations regard at the complexity of the genotype analised.

Key words: DNA, RNA, grapevine

The grapevine, *Vitis vinifera*, has been propagated worldwide and has recently been the subject of genetic investigations. Because wine, a high-value agronomic product, is produced from grapes, it is essential to be able to accurately identify the cultivars (cv.) of vines used for production of specific wines.

Extraction of DNA and RNA from *Vitis* species is difficult because of the abundance of polyphenolics and polysaccharides in their leaves. These contaminants in leaf nucleic acid preparations can render the DNA and RNA resistant to digestion by restriction endonucleases or to PCR amplification [2, 3, 4, 8].

Considering the essential role of nucleic acids in metabolic processes and plant life, we have determined the content of nucleic acids (DNA and RNA) and the ratio among them, in the grapevine variety *Tămâioasă românească* [6,7].

MATERIAL AND METHOD

The biologic material used in the experiment has been represented by *Tămâioasă românească* grapevine variety cultivated in the ampelographic collection of Experimental Didactical Station belonging to University of Agricultural Sciences and Veterinary Medicine from Iasi.

From the relevant sort there were sampled mature leaves from the fertile of shoots from 30 grape vines, in 4 phenophases: unbudding, flowering, ripening up to the full grape maturity. From the relevant leaves 50 mg of mesophyll existing between N1 and N2 nervures was sampled, as close as possible to the leaf stalk.

10 determinations of DNA and RNA content were effected for each phenophases. The extraction of nucleic acids was made after Spirin method [9].

The achieved results for each phenophases have been analyzed from biostatistical point of view [1, 5].

RESULTS AND DISCUSSIONS

The content of nucleic acids

There is ascertained a progressive reduction of DNA content in the mature leaf at the studied grapevine cv. as long as the grape vine progresses in vegetation.

In unbudding phenophase, when the grape vine passes from the resting condition into the vegetation condition, the metabolism is intensified, accelerated bio-synthesis processes are produced so that an increased DNA quantity is evidenced that has a variation ranking between 31.71 mg/g and 32.65 mg/g with an average of 32.05 mg/g (*tab. 1, fig. 1*).

A proper nitrogen feeding causes a normal growing as well as a proper colouring of the leaf that allows the achievement of photosynthesis in the most favourable conditions and at the same time, the differentiation of floriferous buds is favoured. Therefore, at the flowering process, the quantity of DNA is relatively increased but it decreases significantly in the unbudding process, the average being of 8.73 mg/g (*tab. 1, fig. 1*).

By the end of the active vegetation period a quantitative decrease of DNA is noticed, so that in grapes ripening phenophase DNA reaches 6.15 mg/g (*tab. 1, fig. 1*).

At the full grape maturity, the quantity of DNA it grows very easily until 4.85 mg/g (*tab. 1, fig. 1*).

As concerns the quantity of RNA it was much diminished compared to the quantity of DNA at all phenological phases of vegetation period (*tab. 2, fig. 1*).

At unbudding, the amount of RNA is 9.36 mg/g. In this case, too, a significant decrease of RNA amount was registered, since the phenological phase of flowering, when the mean value was 1.86 mg/g. At ripening, the RNA decreases until 1.55 mg/g, so that at full maturation of grapes, it increases until 1.66 mg/g.

As concerns the variability of the content of nucleic acids, we found out that in case of DNA, this parameter was low at phenological phases of unbudding, higher at flowering, and moderate at ripening and grape full maturity (*tab. 1, fig. 2*). In case of RNA, the variability coefficient is higher compared to DNA, at most of phenological phases of the vegetation period. The variability of RNA content is

moderate only at unbudding, higher at flowering and ripening, high degree at full grape maturity (*tab. 2, fig. 2*).

Table 1

The DNA content in the mature leaves of *Tămâioasă românească* grapevine variety

Phenophase	The average value (mg/g)	Standard deviation (S) (mg/g)	Variability coefficient (S%)
Unbudding	32.05	0.632	1.97
Flowering	8.73	2.336	26.76
Ripening	6.15	0.933	15.17
Full grape maturity	4.85	0.613	12.64

Table 2

The RNA content in the mature leaves of *Tămâioasă românească* grapevine variety

Phenophase	The average value (mg/g)	Standard deviation (S) (mg/g)	Variability coefficient (S%)
Unbudding	9.36	1.089	11.63
Flowering	1.86	0.649	34.89
Ripening	1.55	0.402	25.94
Full grape maturity	1.66	0.705	42.47

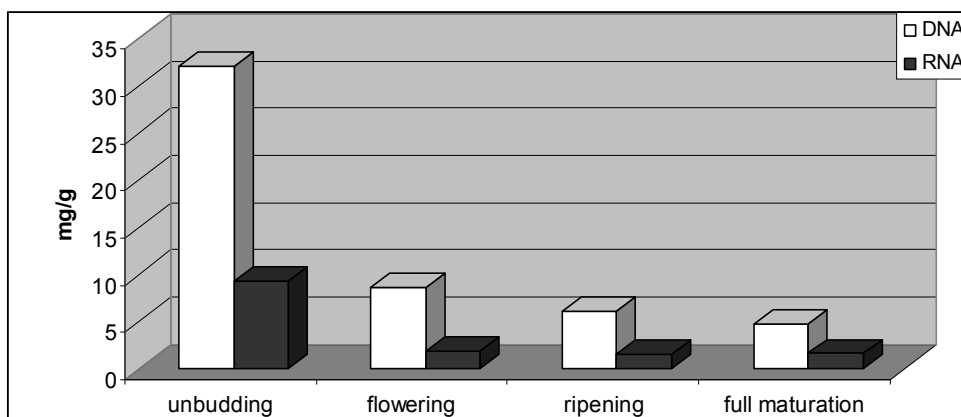


Figure 1 The DNA and RNA content in mature leaves of *Tămâioasă românească* grapevine variety

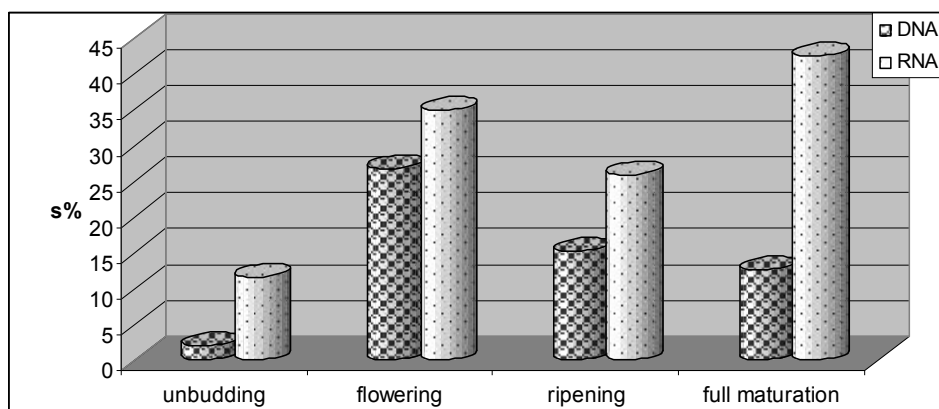


Figure 2 The variability of DNA and RNA content in mature leaves of *Tămâioasă românească* grapevine variety

The limit differences of the content of nucleic acids in the mature leaf of the *Tămâioasă românească* vine variety are presented in tables 3 and 4.

Table 3
The DNA content in the mature leaves of *Tămâioasă românească* grapevine variety

Phenophase	The average value (mg/g)	Difference by comparison control	Significance of difference
Average (control)	12.95	-	-
Unbudding	32.05	+ 19.1	***
Folwering	8.73	- 4.22	00
Ripening	6.15	- 6.80	000
Full grape maturity	4.85	-8.10	000
DL5%=1.998, DL1%=2.873, DL 0.1% = 4.225			

Table 4
The RNA content in the mature leaves of *Tămâioasă românească* grapevine variety

Phenophase	The average value (mg/g)	Difference by comparison control	Significance of difference
Average (control)	3.61	-	-
Unbudding	9.36	+ 5.75	***
Folwering	1.86	- 1.75	0
Ripening	1.55	- 2.06	00
Full grape maturity	1.66	- 1.95	0
DL 5% = 1.367, DL 1% = 1.966, DL 0.1% = 2.891			

In figure 3 we present the amount of total nucleic acids at all the four phenological phases of vegetation. We found out that the maximum value was registered at unbudding, and the minimum one at full grape maturity. A significant difference was found between the amount of total nucleic acids, present at unbudding, compared to the other phenological phases.

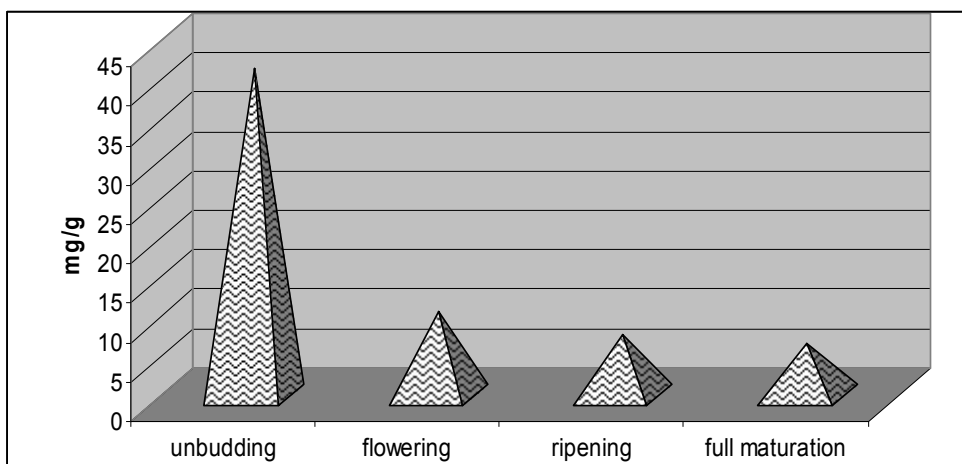


Figure 3 **Total nucleic acids in mature leaves of *Tămâioasă românească* grapevine variety**

RNA/DNA ratio

The DNA amount in the vegetal cell is relatively constant, within certain limits, because of the cell functional state, while the RNA amount is variable. On the basis of the assessment of ratio between RNA and DNA amounts in the leaves of vine varieties, one can make observations on the complexity of the genetic material of noble vine varieties.

In *Tămâioasă românească* grapevine variety, the RNA/DNA ratio is 0.29 at unbudding, 0.21 at flowering, 0.25 at ripening and 0.34 at full grape maturity (fig. 4). We noticed that the RNA/DNA ratio varied in restricted limits within the studied genotype, during the phenological phases.

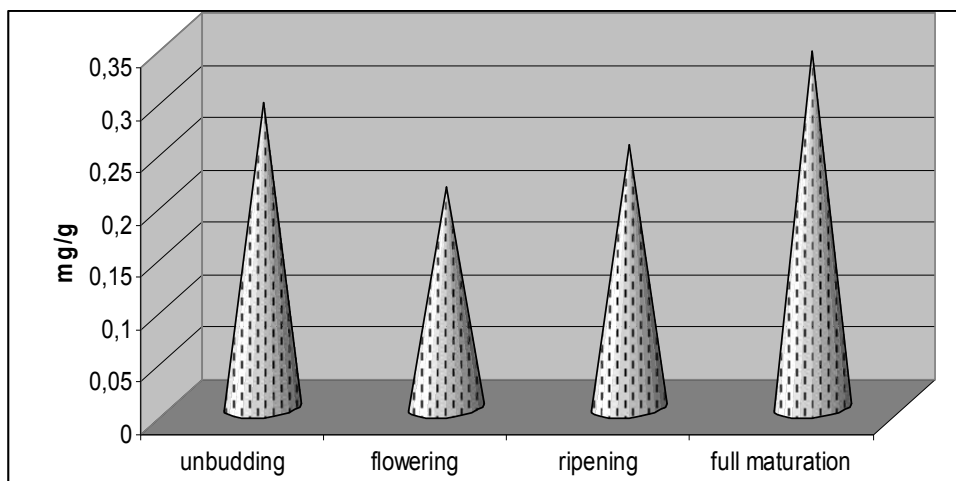


Figure 4 **RNA/DNA ratio in mature leaves of *Tămâioasă românească* grapevine variety**

CONCLUSIONS

1. According to the experimental results, the differences between DNA and RNA amounts at each phenological phase of the vegetation period determine the phenotypical and adaptative characteristics of the grapevine variety *Tămâioasă românească* (variety with middling growth vigour, with a relatively low percentage of fertile shoots, with a short vegetation period - 160-170 days, with resulting in getting high quality flavoured wines).

2. Both the DNA and RNA amounts are maximum ones at the unbudding phenological phase. The quantity of total nucleic acids is maximum one at unbudding and minimum at full grape maturity.

3. Beginning with the phenological phase of flowering, the quantity of nucleic acids decreases significantly until full grape maturation.

4. During the vegetation period, DNA content in the mature leaf is much higher compared to RNA content.

5. The variability of DNA content is more restricted compared to the one of RNA content at the first phenological phases, moderate at ripening and full grape maturity, while at flowering is higher both for DNA and RNA. The variability of RNA content at the ripening also is higher and at full grape maturity, it is high degree.

6. RNA/DNA ratio from mature leaf is maximum at the phenological phase of full grape maturity and minimum at the phenological phases of flowering.

BIBLIOGRAPHY

1. Chițea, G., 2001 – *Biostatistica*, Ed. Univ. Transilvania, Brașov.
2. Fort, F., Hayoun, L., Valls, J., Canals, J. M., Arola, L., Zamora, F., 2007 - *A new and simple method for rapid extraction and isolation of high-quality RNA from grape (Vitis vinifera) berries*, Journal of the Science of Food and Agriculture, Vol. 88 (2), p. 179 - 184.
3. Jinjin, Z., Yuejin, W., Xiping, W., Keqiang, Y., Jinxiao, Y., 2003 - *An improved method for rapidly extracting total RNA from Vitis*, Journal of Fruit Science, 20 (3), p. 178-181.
4. Muhammad, L.A., Ye, G-N, Weeden, N.F., Reisch, B.I., 1994 - *A simple and efficient method for DNA extraction from grapevine cultivars, Vitis species and Ampelopsis*, Plant Molecular Biology Reporter 12(1), p. 6-13.
5. Oancea, S., 2007 – *Ghid de prelucrare rapidă a datelor experimentale*, Ed. Performantica, Iași, p. 14-25, 38-48
6. Pădureanu, S., 2006 – *Variability of RNA content in the leaves of Fetească neagră grapevine sort*, Lucr. st., seria Hort., U.S. A. M. V., Iasi, vol. 49, p. 385-388.
7. Pădureanu, S., 2006 – *Variability of nucleic acids content in the leaves of Fetească albă grapevine sort*, European society for new methods in agriculural research, XXXVI Annual Meeting, Iassy-Romania, p. 553-558.
8. Reid, K. E., Olsson, N., Schlosser, J., Peng, F., Lund, S.T., 2006 - *An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development*, Plant Biology, vol. 6, p. 27.
9. Spirin, A., 1958 – *Spektrofotometricheskoe opredelenie summarnovo kolicestva nucleinovich kislot*, Biohimia, p. 656.