NARDI FUNDULEA, ROMANIA
www.incda-fundulea.ro

ROMANIAN AGRICULTURAL RESEARCH, NO. 35, 2018
Print ISSN 1222–4227; Online ISSN 2067–5720

# GENETIC DIVERSITY OF *TaSAP1-A1* LOCUS AND ITS ASSOCIATION WITH TKW IN SOME EUROPEAN WINTER WHEAT CULTIVARS

**Daniel Cristina[1, 2*], Matilda Ciucă[2], Vasile Mandea[2], Călina-Petruţa Cornea[1]**

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]National Agricultural Research and Development Institute Fundulea, 915200 Fundulea, Călăraşi County, Romania
[*]Corresponding author. E-mail: danielcristina89@gmail.com

## ABSTRACT

**Stresses associated proteins (SAPs), a fast emerging class of zinc-finger proteins (ZFPs), are composed of special types (A20/AN1) of ZFP domains and were identified for the first time in rice as products of multiple stress responsive genes. SAPs are potential candidates for biotechnological approaches in order to improve abiotic stress tolerance in plants - the ultimate aim of which is crop-yield protection. In wheat there is a member of the stress association protein (SAP) gene family, named *TaSAP1-A1* (located on chromosome 7A), involved in response to several abiotic stresses including drought, salt and cold.**

**We analysed 33 European winter wheat cultivars using the markers T7AM5, T7AM2606 and T7AM39, developed by Chang et al. (2013), and located in the promoter region of *TaSAP1-A1* gene. Compared to Chang study, our analysis revealed different sizes for the PCR products obtained with T7AM5 and T7AM2606 markers. Furthermore, digestion of PCR product obtained with T7AM5 marker revealed the existence of a new allelic variant (present in four cultivars). Results showed that *Hap II* is the main haplotype, present in 18 cultivars (55%). *Hap II* was found to be significantly associated ($p<0.05$) with thousand kernel weight (TKW).**

**Key words:** wheat, TaSAP1, abiotic stress, molecular markers.

## INTRODUCTION

The ability of agriculture to meet the current and future demands for food, feed, fibre, and fuel is shaped by the population growth, arable land, fresh water limits, and climate change. It is estimated that the world population will reach 9.1 billion by 2050, 34 percent higher than today. Urbanization will continue at an accelerated pace, and about 70 percent of the world's population will be urban (compared to 49 percent today). The amount of arable land has not changed significantly in the past years, and losses from urbanization, salinization and desertification are not good news for the future of agriculture. Water scarcity is already a critical concern in parts of the world (Fedoroff, 2010; http://www.fao.org/.../How_to_Feed_the_World_in_2050.pdf).

Constantly changing environments are often unfavourable or stressful for plant growth and development. Plants have to overcome several environmental stresses for survival. Environmental conditions include biotic stress, such as pathogen infection and herbivore attack, and abiotic stress, such as drought, heat, cold, nutrient deficiency, and excess of salt or toxic metals. Drought, salt, and temperature stresses are major environmental factors that affect the geographical distribution of plants in nature, limit plant productivity in agriculture, and threaten food security (Boyer, 1982; Zhu et al., 2016). In order to protect themselves against biotic and abiotic stresses plants continuously sense and respond to their surrounding environment. Such stress response mechanisms involve perception of a stimulus, transmission of the signal from one molecule to another spatially and activation of terminal components (Giri et al., 2013).

Wheat continues to be one of the most important crop cultivated worldwide, alongside with rice and maize directly providing about 50% of human food calories, annual increase of 1.6-2% in grain yield is

Received 10 January 2018; accepted 12 February 2018. First Online: February, 2018. DII 2067-5720 RAR 2018-37

required in the coming years in order to fulfil the global demand (Patil, 2013; Faris, 2014). To achieve higher yields in wheat, breeding programs focused on obtaining cultivars with best agronomic traits (disease resistance, protein content, grain size and weight, drought tolerance etc.). This achievement can be made through genetic improvements based on enhanced plant biology understanding.

Stress associated proteins (SAPs), a fast emerging class of zinc-finger proteins (ZFPs), show significant levels of structural conservation and functional similarity among diverse plant species. These proteins are composed of special types (A20/AN1) of ZFP domains and were identified for the first time in rice as products of multiple stress responsive genes (Giri et al., 2013). At present there are 18 SAP genes identified in rice, 14 in Arabidopsis, 13 in tomato, and 11 in maize (Giri et al., 2013; Zhang et al., 2015).

The first plant A20/AN1 protein identified, namely OsSAP1, showed enhanced expression in response to multiple stresses such as salt, drought, cold, submergence, mechanical wounding, abscisic acid (ABA) and membrane fluidifiers (Mukhopadhyay et al., 2004; Giri et al., 2013). Another characterized SAP, *ZFP177* (*OsSAP9*) is expressed constitutively in rice leaves, culms, roots and spikes and its expression was enhanced by cold, heat and $H_2O_2$ stress, but repressed by salt stress; *ZFP177* remained unaffected under drought stress. Similarly, maize and Arabidopsis SAP genes responded to cold, salt, osmotic, and drought stresses in a tissue and stress-specific manner.

Almost all the SAPs studied so far show positive effects on tolerance to one or other abiotic stresses in plants at various growth stages. The physiological phenomenon of improved stress tolerance is manifested together with an agriculturally desirable trait–protection of crop-yield loss under stress conditions (Giri et al., 2013).

*TaSAP1-A1* (A20/AN1; located on chromosome 7A), *TaSAP7-B* (A20/AN1; located on chromosome 5B) and *TaSAP17-D* (AN1/AN1) are members of the stress associated protein (SAP) gene family from wheat. Previous studies showed that these genes are involved in response to several abiotic stresses, including drought, salt and cold (Chang et al., 2013; Wang et al., 2017; Xu et al., 2017).

Chang et al. (2013) developed two CAPS markers T7AM5, T7AM2606 and an allele-specific PCR marker T7AM39 based on three selected variations in the promoter region of *TaSAP1-A1*, including a 5 bp indel at position -1,810 bp (InDel5-1810), a SNP (A–C) at position -2,606 bp (SNP-2606) and a 39 bp indel at position -1,637 bp (InDel39-1637). A total of six haplotypes were identified, haplotypes that significantly affected the agronomic traits, including thousand kernel weight (TKW), spike length, total number of spikelets per spike, number of grains per spike and peduncle length.

Chang et al. (2013) found in their study that the average TKW of Hap III was significantly different ($p<0.05$) from those of Hap I an IV in almost all environments. Also, significant differences ($p<0.05$) were identified in three drought-stress regimes between Hap II and Hap I. For peduncle length, Hap IV and II were longer than Hap III and I in all environments, with a significant difference ($p<0.05$) between Hap II and I.

The objective of this study was to evaluate the genetic diversity of *TaSAP1-A1* locus in some European winter wheat cultivars and the association of haplotypes with TKW and/or TKW components.

## MATERIAL AND METHODS

**Plant material** was obtained from NARDI Fundulea, Romania and consisted of 33 European winter wheat cultivars, tested in the experimental Fundulea field (Table 1).

**Phenotypic data** contain the length and width of kernels, TKW and FFD (factor form-density), described as FFD = TKW/length * width (Giura and Saulescu, 1996), averaged over three years (2013, 2014 and 2015).

*Table 1*. Analysed cultivars and molecular results for *TaSAP-A1*

| Cultivar | Pedigree | Released | Length (mm) | Width (mm) | FFD | TKW (g) | T7AM 2606 | T7AM 39 | T7AM 5 | *TaSAP-A1* |
|---|---|---|---|---|---|---|---|---|---|---|
| Diana | Fiorello/Bezostaya1 | 1976 | 7.15 | 3.20 | 1.997 | 45.7 | A | - | + | II |
| Ariesan | Rubin/T141 | 1985 | 7.87 | 3.34 | 1.720 | 45.2 | A | - | + | II |
| Lovrin 231 | BEZOSTAYA-1/FIORELLO | 1974 | 7.31 | 3.30 | 1.864 | 44.8 | H | + | - | I-VI |
| Dropia | COLOTANA/2120W1 | 1993 | 6.65 | 3.40 | 1.981 | 44.7 | A | - | + | II |
| Transilvania 1 | US (60)43/Avrora//T141-65 | 1981 | 7.36 | 3.22 | 1.833 | 43.0 | C | + | + | III |
| Glosa | F135U2-1/F508U1-1BUCUR | 2005 | 6.72 | 3.38 | 1.867 | 42.5 | A | - | + | II |
| Flamura 85 | JUWEL/Lv32A//2*FL80 | 1989 | 6.54 | 3.27 | 1.963 | 41.9 | A | - | + | II |
| Boema 1 | F308O2-20/DROPIA | 2000 | 6.60 | 3.37 | 1.877 | 41.8 | A | - | + | II |
| Renesansa | YUGOSLAVIA/NS-55-25 | 1995 | 6.66 | 3.40 | 1.821 | 41.3 | A | - | + | II |
| Ceres | Michiurinka/Bezostaya1 | 1974 | 6.69 | 3.28 | 1.863 | 40.8 | H | + | + | III-V |
| Pitar | 02555-GP-2/00099-GP-2 | 2015 | 6.34 | 3.33 | 1.941 | 40.7 | A | - | + | II |
| Fundulea 4 | Fundulea29/2*Lovrin32 | 1994 | 6.60 | 3.19 | 1.889 | 39.9 | A | - | + | II |
| Pajura | IZVOR/F96012G2-2//GLOSA | 2014 | 6.51 | 3.37 | 1.803 | 39.7 | A | - | + | II |
| Bezostaya 1 | LUTESCENS-17 /SKOROSPELKA-2 | 1959 | 6.65 | 3.13 | 1.891 | 39.4 | C | + | + | III |
| Dacia | Bucuresti1/Skorospelka3 | 1971 | 6.06 | 3.29 | 1.972 | 39.4 | C | - | + | IV |
| Iulia | Bezostaya1/Beloterkovsk198 | 1974 | 6.58 | 3.13 | 1.890 | 39.1 | A | - | + | II |
| Izvor | KARL/F201R2-111//F508U1-1 | 2008 | 6.26 | 3.34 | 1.855 | 38.9 | C | - | + | IV |
| Litera | ERYT26221/F96869G1-1// GLOSA | 2009 | 6.61 | 3.35 | 1.734 | 38.4 | A | - | + | II |
| F132 | 20E3-22/FL80//553D4-22 | - | 6.33 | 3.02 | 1.988 | 38.0 | A | - | + | II |
| Exotic | ET.CHOISY/VIVANT | 2005 | 6.28 | 3.33 | 1.805 | 37.8 | C | + | - | I |
| Miranda | ERYT26221/96869G1-1 //GLOSA | 2010 | 6.45 | 3.14 | 1.837 | 37.4 | A | - | + | II |
| F628 | 191TR1-122/Bucur//PL | - | 6.81 | 3.37 | 1.613 | 37.0 | C | + | - | I |
| Alex | Flamura80/Fundulea29 | 1994 | 6.86 | 3.26 | 1.639 | 36.9 | A | - | + | II |
| Otilia | F96052G16-2/FAUR | 2014 | 6.01 | 3.26 | 1.876 | 36.8 | A | - | + | II |
| Capo | POKAL/MARTIN | 1989 | 6.07 | 3.14 | 1.910 | 36.4 | C | + | - | I |
| Jagger | KS-82-W-418/STEPHENS | 1994 | 6.22 | 2.95 | 1.924 | 35.3 | H | + | - | I-VI |
| Odesskaya 51 | Odesskaya16/Bezostaya4 | 1969 | 6.23 | 3.16 | 1.793 | 35.2 | C | - | + | IV |
| A15 | Sel Tenmarq | 1933 | 6.46 | 3.04 | 1.777 | 34.9 | H | + | - | I-IV |
| Fundulea 133 | SWW7/PRIBOI | 1985 | 5.97 | 3.00 | 1.934 | 34.7 | C | - | + | IV |
| Fundulea 29 | Aurora/Riley67 | 1979 | 6.35 | 3.15 | 1.692 | 33.9 | A | - | + | II |
| Apache | AXIAL/NRPB-84-4233 | 1998 | 6.29 | 3.22 | 1.671 | 33.8 | C | + | + | III |
| Doina | Et.Choisy/Monon | 1977 | 6.21 | 3.23 | 1.680 | 33.7 | A | - | + | II |
| Chinese Spring | | | 5.23 | 2.87 | 2.072 | 31.1 | C | + | - | I |

**DNA extraction** was performed on two dry seeds using SDS3 method (Cristina et al., 2017).

**DNA amplification** was performed with the PCR kits "MyTaq Mix" and "MyTaq Red DNA Polymerase" from Bioline in an ABI ProFlex™ 3 x 32-well PCR System.

PCR parameters for the amplification with T7AM5 marker, using MyTaq Red DNA Polymerase kit, were as follows: 15 µL final reaction volume containing 1X reaction buffer, 0.5 mM primers, 0.5U DNA polymerase and 60-80 ng DNA sample. PCR programme: initial denaturation at 95°C for 1 min, followed by 35 cycles: 95°C – 15 s, 58°C – 15 s, 72°C – 15 s, and a final extension at 72°C for 5 min. Next, the PCR products were subjected to digestion with *HhaI* restriction enzyme (Promega).

PCR parameters for the amplification with T7AM2606 and T7AM39 markers (MyTaq Mix kit) were as follows: 15 µL final reaction volume containing 1X reaction buffer, 0.5 mM (0.6 mM for T7AM39) primers and 70-80 ng DNA sample. PCR programme: initial denaturation at 95°C for 2 min, followed by 35 cycles: 95°C – 15 s, 60°C (61°C for T7AM39) – 15 s, 72°C – 25 s, and a final extension at 72°C for 5 min. The PCR products obtained with T7AM2606 were also digested with *HhaI*.

**Gel electrophoresis** for the PCR products and the digested PCR products were carried out with routine use agarose stained with ethidium bromide and visualized on UV light.

Gels concentration were as follows: 1.2% agarose gel for T7AM39 PCR products, 1.5% for the digested products obtained with T7AM2606/HhaI and 2% for the digested PCR product obtained with T7AM5/HhaI.

**Haplotypes,** of *TaSAP-A1* gene, were determined according to Chang et al. (2013) (Figure 1).

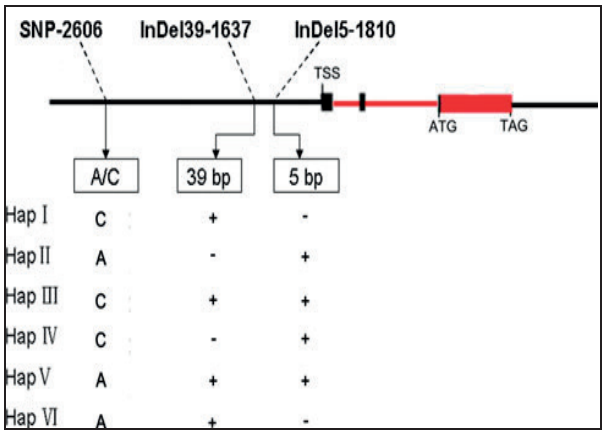


*Figure 1. TaSAP-A1* haplotypes

**Statistical analysis** (t-test) was performed using the online tools available at: http://www.socscistatistics.com/tests/studentttest/Default2.aspx and http://www.evanmiller.org/ab-testing/t-test.html.

## RESULTS AND DISCUSSION

Exploitation and utilization of superior genes and allelic variations can be helpful approaches for improving wheat production. Enormous amount of allelic variations is present in wheat germplasm. Association analysis has the advantages of shorter research time and higher mapping resolution, therefore, it is considered as a powerful approach for identifying superior allelic variations.

In our study PCR with T7AM5 marker amplified a fragment of ~1000 bp. After the digestion with *HhaI,* genotypes with the 5 bp insertion presented the following fragments: 800 bp + 196 bp, 800 bp + 230 bp or 800 bp + 230 bp + 196 bp.

These results suggest the existence of a new allelic variant generated by a different position of the restriction site of *HhaI* (cultivars Apache and Transilvania 1). Also, a secondary restriction site of *HhaI* was observed in case of cultivars Ceres and Bezostaya 1 suggesting the existence of a heterozygous/ heterogenic form (Figure 2).

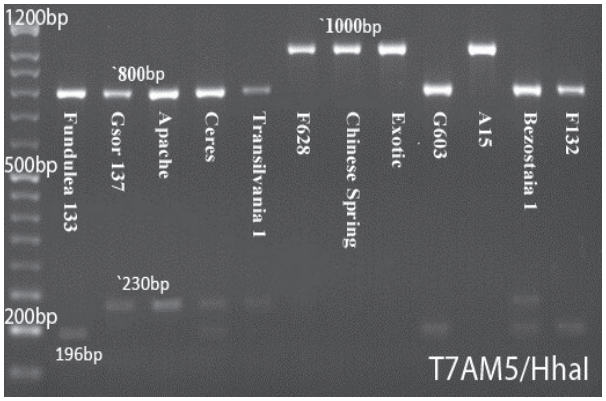


*Figure 2.* Electrophoretic pattern obtained with T7AM5/*HhaI*

In this paper the haplotypes for *T7AM5* were noted according to Chang's paper, thereby, the cultivars with *HhaI* restriction site were considered as positive (+) for the 5 bp insertion, and the cultivars without the *HhaI* restriction site as negative (-) (Figure 3).

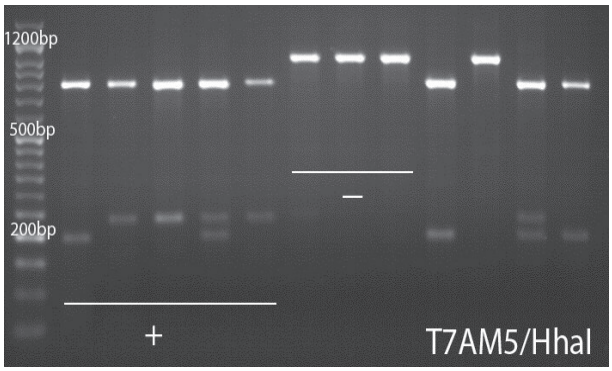


*Figure 3. T7AM5/HhaI* haplotype notation

PCR with T7AM2606 marker also amplified a fragment of ~1000 bp. After the digestion with *HhaI* two fragments of 860 + 140 bp resulted in genotypes with SNP-2606**C**, undigested fragment of ~1000 bp for genotypes with SNP-2606**A.** Heterozygous/ heterogenic genotypes were also present and noted as "**H**" (Figure 4).

PCR with T7AM39 marker amplified a ~1906bp fragment in genotypes with the 39bp insertion representing the positive haplotype (+). Genotypes without the 1906 bp fragment were noted as negative (-) (Figure 5).

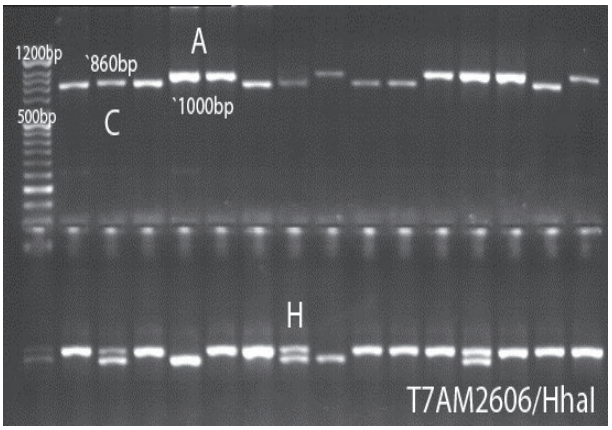


*Figure 4.* Electrophoretic pattern obtained with T7AM2606/*HhaI*

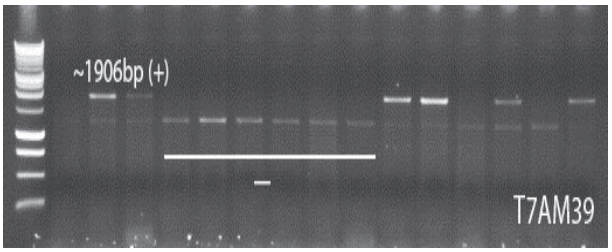


*Figure 5*. Electrophoretic pattern obtained with T7AM39 marker

Molecular results are presented in Table 1 (cultivars were sorted by TKW values). Hap II was the main haplotype found in 18 cultivars, followed by Hap I, Hap IV, mixed haplotypes and Hap III. *TaSAP-A1* haplotype distribution is represented in Figure 6.

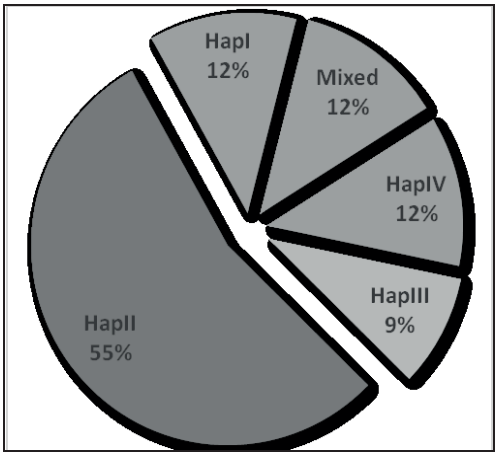


*Figure 6. TaSAP-A1* haplotype distribution

**Statistical analysis.** The presence of heterozygous/ heterogenic genotypes revealed by the T7AM2606 marker lead to mixed haplotypes for *TaSAP-A1* (cultivars A15, Ceres, Jagger and Lovrin 231), therefore, these cultivars were removed from the statistical analysis.

Mean values for the phenotypic data are presented in Table 2.

*Table 2.* Mean phenotypic data values

| Haplotype | Length (mm) | Width (mm) | FFD | TKW (g) |
|---|---|---|---|---|
| Hap I | 6.10 | 3.18 | 1.85 | 35.58 |
| **Hap II** | 6.61 | **3.27** | 1.84 | **39.87** |
| Hap III | **6.77** | 3.19 | 1.80 | 38.73 |
| Hap IV | 6.13 | 3.20 | **1.89** | 37.03 |
| Mix (heterozygous/ heterogenic) | 6.67 | 3.14 | 1.86 | 38.97 |
| I+III+IV (Non-Hap II) | 6.29 | 3.19 | 1.85 | 36.97 |
| I+III+IV+Mix | 6.39 | 3.18 | 1.85 | 37.50 |

The t-test analysis performed on the results revealed significant association ($p<0.05$) of haplotype determined by SNP-2606A, respectively, Hap II of *TaSAP-A1* with TKW. No other association could be found between our phenotypic data and *TaSAP-A1* haplotypes (Table 3).

*Table 3*. Association of haplotypes with phenotypic data

| | Hap II | Stdev. | Non-Hap II | Stdev. | p-value |
|---|---|---|---|---|---|
| Width (mm) | 3.27 | 0.108 | 3.19 | 0.150 | 0.12183 |
| Length (mm) | 6.61 | 0.400 | 6.29 | 0.537 | 0.07645 |
| FFD | 1.84 | 0.112 | 1.85 | 0.130 | 0.90097 |
| **TKW (g)** | 39.87 | 3.491 | 36.97 | 3.253 | **0.035006** |

Hap II TKW ranged between 33.7 g and 45.7 g, 8 out of 18 cultivars had TKW over 40 g. TKW of Non-Hap II haplotype ranged between 31.1 g and 43 g, most cultivars being in 36-40 g TKW group.

Frequency and normal distributions of TKW for Hap II and Non-Hap II haplotypes are presented in Figure 7.

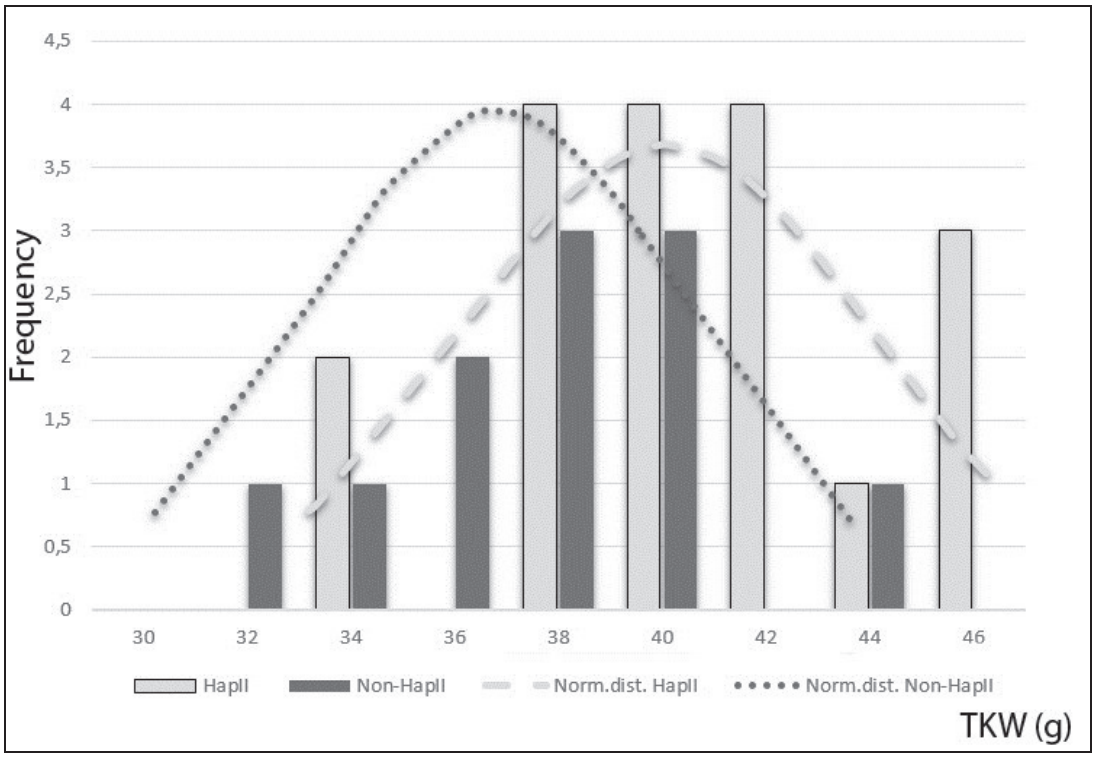


*Figure 7.* Frequency and normal distributions of TKW for Hap II and Non-Hap II haplotypes

Considering the small number of cultivars analysed in this study, future research will focus on a higher number of genotypes in order to validate the association of Hap II/ SNP-2606A with TKW. Also, the new allelic variant obtained with T7AM5 marker will be analysed for association with agronomical traits.

## CONCLUSIONS

Genetic diversity analysis of 33 European winter wheat cultivars, at *TaSAP-A1* locus, revealed the presence of Hap II/SNP-2606A in 18 cultivars representing 55% of the analysed cultivars, followed by Hap I (12%), Hap IV (12%), mixed haplotypes (12%) and Hap III (9%).

Statistical analysis showed a significant association ($p<0.05$) of Hap II/ SNP-2606A with TKW. No other associations with our phenotypic data could be found.

Marker T7AM5 revealed a new allelic variant. Further research on a higher number of genotypes will be conducted to verify if the new allelic variant is associated with desirable agronomic traits.

## ACKNOWLEDGEMENTS

The present work was funded through the Ministry of Agriculture and Rural Development – ROMANIA, Research Project ADER116 (2015-2018).

## REFERENCES

Boyer, J.S., 1982. *Plant productivity and environment*. Science, 218(4571): 443-448.

Chang, J., Zhang, J., Mao, X., Li, A., Jia, J., & Jing, R., 2013. *Polymorphism of TaSAP1-A1 and its association with agronomic traits in wheat*. Planta, 237(6): 1495-1508.

Faris, J.D., 2014. *Wheat domestication: Key to agricultural revolutions past and future*. In: Genomics of plant genetic resources: 439-464, Springer Netherlands.

Fedoroff, N.V., Battisti, D.S., Beachy, R.N., Cooper, P.J.M., Fischhoff, D.A., Hodges, C.N., Knauf, V.C., Lobell, D., Mazur, B.J., Molden, D., Reynolds, M. P., Ronald, P. C., Rosegrant, M. W., Sanchez, P.A., Vonshak, A., and Zhu, J.-K., 2010. *Radically rethinking agriculture for the 21st century*. Science, 327(5967): 833-834.

Giri, J., Dansana, P.K., Kothari, K.S., Sharma, G., Vij, S., & Tyagi, A.K., 2013. *SAPs as novel regulators of abiotic stress response in plants*. Bioessays, 35(7): 639-648.

Giura, A. and Saulescu, N.N., 1996. *Chromosomal location of genes controlling grain size in a large grained selection of wheat (Triticum aestivum L.)*. Euphytica, 89: 77-80.

*How to Feed the World in 2050*. Available online at: http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf (last accessed: 05.02.2018).

Mukhopadhyay, A., Vij, S., & Tyagi, A.K., 2004. *Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco*. Proceedings of the National Academy of Sciences of the United States of America, 101(16): 6309-6314.

Patil, R.M., Tamhankar, S.A., Oak, M.D., Raut, A.L., Honrao, B.K., Rao, V.S., Misra, S.C., 2013. *Mapping of QTL for agronomic traits and kernel characters in durum wheat (Triticum durum Desf.)*. Euphytica, 190, 1: 117-129.

Wang, Y.X., Xu, Q.F., Chang, X.P., Hao, C.Y., Li, R.Z., & Jing, R.L., 2017. *A dCAPS marker developed from a stress associated protein gene TaSAP7-B governing grain size and plant height in wheat*. DOI: 10.1016/S2095-3119(17)61685-X.

Xu, Q.F., Mao, X.G., Wang, Y.X., Wang, J.Y., Xi, Y.J., & Jing, R.L. 2017. *A Wheat Gene TaSAP17-D encoding an AN1/AN1 zinc finger protein improves salt stress tolerance in transgenic Arabidopsis.* Journal of Integrative Agriculture, 2017, 16(0): 60345-7.

Zhang, Y., Lan, H., Shao, Q., Wang, R., Chen, H., Tang, H., Zhang, H., & Huang, J., 2015. *An A20/AN1-type zinc finger protein modulates gibberellins and abscisic acid contents and increases sensitivity to abiotic stress in rice (Oryza sativa).* Journal of Experimental Botany, 67(1): 315-326.

Zhu, J.K., 2016. *Abiotic stress signaling and responses in plants*. Cell, 167(2): 313-324.