SCREENING WINTER WHEAT GERMPLASM FOR DETECTION OF 1-FEH W3 VARIANTS FOR IMPROVEMENT OF DROUGHT TOLERANCE USING KASP ASSAY

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Abstract

Wheat yield levels and stability are endangered by drought, which is one of the main effects of current climate changes. A possible way of increasing wheat yield under water stress could be the remobilization of stem assimilates for grain filling. *1-FEH w3* (*1-FEH-6B*) is a key enzyme involved in stem water-soluble carbohydrates (WSC) remobilization, playing an important role during grain filling under drought stress. The objective of this study was the screening of a winter wheat collection from NARDI Fundulea regarding the *1-FEH w3* haplotypes using Kompetitive Allele Specific PCR (KASP) SNP marker. KASP genotyping assay on 64 wheat genotypes (cultivars, breeding and pre-breeding lines) showed that 25 genotypes carried the "Kauz" type susceptible haplotype (K) and 39 genotypes carried the "Westonia" type haplotype (W), considered the favorable haplotype in drought conditions. The favorable haplotype (W) was found in several cultivars known for their good performance under water stress (such as Fundulea 133, or A15), but also in some cultivars with poor performance under drought (such as Apache, Ariesan or Bezostaya 1), which suggests that *1-FEH w3* is not the only factor determining drought response. On the other hand, the haplotype associated with water stress susceptibility was found in cultivars known as drought resistant (such as Izvor or Dropia), suggesting that the performance of these cultivars under drought might be further improved by incorporating by breeding the favorable variant of the *1-FEH w3* gene. These results open perspectives of breeding for improved drought resistance by pyramiding several favorable alleles for response to water stress.

Key words: 1-FEH, wheat, drought tolerance, WSC, KASP

Wheat is one of the major crops worldwide and grain yield is one of the major objectives in wheat breeding programs. Among the limiting factors, droughts have severely impacted grain yields in recent decades. Droughts are expected to become more severe and frequent as a result of global warming, becoming a greater threat to wheat production in many parts of the world (Fatima I. *et al*, 2021).

Drought affects wheat performance at all stages of growth, but it is most severe during the flowering and grain-filling periods, leading to substantial yield losses. Drought tolerance in wheat has been linked to high levels of stem water-soluble carbohydrates (WSC).

Mobilization of WSC during grain filling can potentially contribute about 20% of the final grain weight under non-stress conditions, and up to 70% or more of grain dry matter under drought stress in wheat (Goggin D.E., Setter T.L., 2004).

WSC in wheat stems are mainly composed of fructan, sucrose, glucose, and fructose, in with fructan is the major component at the late stage of WSC accumulation phase (Nadia K. *et al*, 2017).

1-FEH w3 (*1-FEH-6B*) is a major contributor involved in stem fructan remobilization process playing an important role during grain filling under drought stress. In Zhang J. *et al* (2015) study, the Westonia genotype was linked to high gene *1-FEH w3* expression and high TGW indicating that the high gene expression of *1-FEH w3* contributed to the high levels of the stem WSC remobilization. Also, the harvest results presented in this study showed that the TGW in Westonia type were consistently higher than in Kauz type.

Based on a single nucleotide polymorphism (SNP) detected in an auxin response element in the 1-FEH w3 promoter region, a cleaved amplified polymorphic (CAP) marker was designed by Zhang J. *et al* (2015) and a corresponding Kompetitive allele specific PCR (KASP) marker was designed by Rasheed A. *et al* in 2016.

SNP-based assays have revolutionized the method of genotyping as these consume less time and have high accuracy and effectiveness. KASP is

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a homogenous, fluorescence-based genotyping variant of polymerase chain reaction. It is a simple, fast and economical method that allows a high-precision bi-allelic characterization of SNPs, as well as insertions and deletions in specific loci. The KASP method introduces fluorescence resonance energy transfer (FRET) for signal generation, where two fluorescent cassettes are used for the identification of allele-specific amplification for a single bi-allelic SNP.

In this study a collection of 64 winter wheat cultivars, breeding and pre-breeding lines from NARDI Fundulea were screened for the *1-FEH w3* haplotypes using Kompetitive Allele Specific PCR (KASP) SNP marker.

MATERIAL AND METHOD

The plant material consisted of 64 winter wheat cultivars (cultivars, breeding and pre-breeding lines) grown at NARDI Fundulea, Romania (*table 1*).

DNA amplification was carried out in an Eppendorf Mastercycler ep Gradient S and the plate fluorescent readings were performed in a FLUOstar Omega Microplate Reader (BMG LABTECH). The genotyping data analysis and reporting was processed with KlusterCaller software (LGC, Biosearch Technologies).

The primer mix was ordered from LGC - Biosearch Technologies, based on the SNP sequence (*table 1*). Primers carry a standard FAM tail (5′-GAAGGTGACCAAGTTCATGCT-3′) and HEX tail (5′-GAAGGTCGGAGTCAACGGATT-3′) with different fluorescence signals.

Table 1

SNP sequence and KASP primers sequences for 1-FEH w3

SNP sequence	CTATACATCCTATCGCTCTCC TCCCCCTCCCCCCTTCCTTCT GTC**[T/C]**CCGAGGCCCAAAGC TCGGGCCGTCTTCCTCCTCC TCCTCATCTTCTTCTACAGGA GCGGCGGATCTACAGTC
FAM primer	CTCCCCCCTTCCTTCTGTC**C**
Hex primer	CTCCCCCCTTCCTTCTGTC**T**
Common primer	AGGAAGACGGCCCGAGCTTT

KASP assays were performed in 10.2 µL mixtures containing 2 µL of 30 ng/µL DNA, 5 µL of 1xKASP-TF V4.0 2X Master Mix (KBS-1050-012; LGC-Biosearch Technologies), 0.2 µL of primer mixture, and 3 µL ddH2O and the following amplification programme: denaturation at 95 °C for 15 min, followed by ten touchdown cycles (95 °C for 20 s; touchdown at 61 °C initially and decreasing by 0.6 °C per cycle for 60 s) and 30 additional cycles of annealing (95 °C for 20 s; 55 °C for 60 s).

RESULTS AND DISCUSSIONS

Evolution of bread wheat varieties is driven by multiple factors, particularly many important genetic loci that have been selected during modern wheat breeding. Insight into these genetic loci is important for understanding phenotypic variations in adaptability, resistance to biotic and abiotic stresses, processing and nutritional quality, and yield stability.

In this study, KASP analyses on *1-FEH w3* locus revealed good clustering in KlusterCaller software, resulting in clear differentiation between the two haplotypes, K (Kauz) and W (Westonia) (*figure 1*).

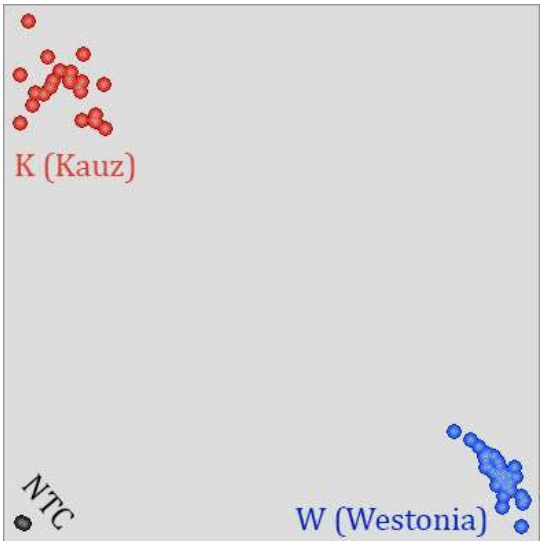

Figure 1 **KASP assay clustering and results for the screening of *1-FEH w3* haplotypes (NTC – no DNA template control)**

Analysis of 64 genotypes using KASP assays revealed that 25 genotypes carry the Kauz type haplotype and the other 39 genotypes carry the Westonia type haplotype (W), considered the favorable haplotype in drought conditions (*table 2*).

Table 2

List of cultivars analyzed in this study and molecular results

No.	Cultivar/line	*1-FEH w3*
1	Ae. Speltoides 21-1	W
2	Ae. Speltoides 21-2	W
3	Ae. Speltoides 21-4	W
4	Ae. Speltoides 22-2	W
5	516-1-I	W
6	574-6	W
7	574-6	W
8	613	W
9	A15	W
10	Abundent	W
11	Alex	W
12	Amurg	K
13	Apache	W

No.	Cultivar/line	*1-FEH w3*
14	Ariesan	W
15	Armura	W
16	Avenue	W
17	Baltag	K
18	Bezostaia	W
19	Boema	K
20	Bogdana	W
21	CGF593	W
22	CGF595	W
23	CS	W
24	Dacia	K
25	Dor	K
26	Dropia	K
27	F628	W
28	Faur	K
29	Fundulea 133	W
30	Fundulea 29	K
31	Fundulea 4	K
32	G557-2	K?
33	GDR 1082	K
34	GGEN 3	W
35	GGEN 33	W
36	GGEN 37	W
37	GGEN 4	W
38	GGEN 65	K
39	GGEN 69	W
40	GGEN 73	W
41	GGEN F	W
42	Glosa	K
43	H175	W
44	H22	K
45	H254	K
46	H255	K
47	H49 POP	W
48	H49G	K
49	H9G	K
50	Iulia	W
51	Izvor	K
52	Lovrin 32	K
53	Miranda	K
54	Otilia	W
55	Pajura	K
56	Pitar	K
57	Renesansa	K
58	SEC	W
59	Semnal	K
60	SP125	W
61	SP61	W
62	Ursita	W
63	Voinic	W
64	Zamfira	W

The favorable haplotype W was found in several cultivars known for their good performance under water stress (such as Fundulea 133, or A15), but also in some cultivars with poor performance under drought (such as Apache, Ariesan or Bezostaya 1), which suggests that *1-FEH w3* is not the only factor determining drought response. On the other hand, the haplotype associated with water stress susceptibility was found in cultivars known as drought resistant (such as Izvor or Dropia), suggesting that the performance of these cultivars under drought might be further improved by incorporating by breeding the favorable variant of the *1-FEH w3*.

In a study published in 2019 by Khalid M. *et al* the allele frequency of *1-FEH w3* was almost equal and the observed favorable haplotype was the Kauz type. Association analysis revealed associations of *1-FEH w3* with grain yield, plant height, and spike length in well watered conditions.

In our previously published study (Cristina D. *et al*, 2021) carried out on 34 wheat cultivars grown in natural conditions between 2013-2015 at NARDI Fundulea, association analysis at *1-FEH w3* locus revealed a significant association only with grain width and the favorable haplotype was also represented by the Kauz type, contrary to Zhang J. *et al* (2015) study.

These results highlight the fact that genetic background of wheat cultivars and interactions with growing environment affect the expression of some genes and/or QTL's. Different favorable haplotypes are observed depending on the region, climate, growing conditions or genetic background of the biological material.

CONCLUSIONS

KASP analysis proved to be a fast, simple and reliable method for screening the *1-FEH w3* locus. Results from this study showed that 25 out of 64 genotypes analyzed carried the Kauz type haplotype while the other 39 genotypes carried the Westonia type haplotype.

Further research is needed on a higher number of genotypes to establish the superior variant of *1-FEH w3* for the Romanian climate and growing conditions. Also, carbohydrate analyses are needed to further validate the effect of stem WSC on yield components in different conditions.

These results open perspectives of breeding for improved drought resistance by pyramiding several favorable alleles for response to water stress.

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REFERENCES

Cristina D., Ciuca M., Mandea V., Cornea C.P., 2021 - *Assessment of 25 genes reported to influence thousand grain weight in winter wheat germplasm*. Cereal Research Communications, pp.1-7.

Fatima I., Gao Y., Xu X., Jin J., Duan S., Zhen W., Xie

C., Ma J., 2021 - *Genome-Wide Association Mapping of Seedling Biomass and Root Traits Under Different Water Conditions in Wheat*. Frontiers in Genetics, 12, p.410.

Goggin D.E., Setter T.L., 2004 - *Fructosyltransferase activity and fructan accumulation during development in wheat exposed to terminal drought*. Functional Plant Biology, 31(1), pp.11-21.

Khalid M., Afzal F., Gul A., Amir R., Subhani A., Ahmed Z., Mahmood Z., Xia X., Rasheed A., He Z., 2019 - *Molecular characterization of 87 functional genes in wheat diversity panel and their association with phenotypes under well-watered and water-limited conditions.* Frontiers in plant science, 10, p.717.

Nadia K., Chang X., Jing R., 2017 - *Genetic dissection of stem water-soluble carbohydrates and agronomic traits in wheat under different water regimes.* J. Agric. Sci, 9, p.42.

Rasheed A., Wen W., Gao F., Zhai S., Jin H., Liu J., Guo Q., Zhang Y., Dreisigacker S., Xia X., He Z., 2016 - *Development and validation of KASP assays for genes underpinning key economic traits in bread wheat*. Theoretical and Applied Genetics, 129(10), pp.1843-1860.

Zhang J., Xu Y., Chen W., Dell B., Vergauwen R., Biddulph B., Khan N., Luo H., Appels R., Van den Ende W., 2015 - *A wheat 1-FEH w3 variant underlies enzyme activity for stem WSC remobilization to grain under drought*. New Phytologist, 205(1), pp.293-305.