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THE INFLUENCE OF VIABILITY ON PHENOLIC CONTENT, CONDUCTIVITY AND SUGARS EFFLUX, AND THE RELATIONSHIPS BETWEEN THESE INDICES IN WHEAT SEEDS

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Abstract. *The influence of wheat seeds viability on phenolic content, conductivity and sugars efflux as well as the relationships between these physiological and biochemical indices was the purpose of this paper. The biological material, supplied by Suceava Agricultural Research and Development Station, was represented by six different seed samples of wheat* (*Triticum aestivum* L. spp. *aestivum*) *belonging to a local cultivar*, *kept different periods in uncontrolled storage conditions. It has determined: seeds viability (germination capacity %) as well as conductivity, transmembrane sugars effllux, Total Phenolic Content (TPC) before germination and TPC post germination, evaluating the relationships between these indices, too. Both before and post germination, the seed samples with higher viability have recorded higher total phenolic content values, compared to samples with low viability. The germination process has increased the total phenolic content values with much higher percentages in samples with risen viability. The correlations between viability and total phenolic content, determined before and post germination, have shown positive correlations significant for relationship Viability - TPC post germination. Regarding the seminal exudate, the conductivity values have fluctuated, the highest value being in seed sample with lowest viability. The sugars efflux have steadily registered increasing values once with seed viability decreasing. The correlation between viability and conductivity, as well as between viability and transmembrane sugars efflux in the six wheat samples, have shown negative correlations, significant for relationship viability - sugars efflux.*

Keywords: *wheat, viability, total phenolic content, conductivity, sugars efflux*

1. Introduction

The seeds viability (germination capacity %) plays an important role in the lives of all plants, where the seed is a new beginning or the life cycle continuing in a new body. But the viability depends on many factors such as: pedo-climatic conditions during seed growth and development, the biocompounds content and quality of these seeds, the coatings integrity (cell membranes), the conditions and duration of grains storage etc. In turn, the viability of seeds can influence physio-biochemical processes in the seed and during plant forming, including the concentration of some nutrients or other compounds (antioxidants) which shows importance for diet and human health. Wheat is an important component of the human diet, and therefore it may be an important source of phenolic antioxidants [1], a wide variation of phenolic content being in wheat cultivars [2]. Important macronutrient sources that contain a wide range of antioxidant compounds are especially whole-grain cereals [3]. According to Hyun Young Kim et al.

(2013), cereals are rich in phenolic compounds, vitamin E, γ-oryzanol, and carotenoids, and contain small quantities of phytoestrogens and flavonoids. In this paper it has searched in what extent the viability can influence the phenolic content, conductivity and sugars efflux in wheat seeds, as well as the relationships between these physiological and biochemical indices.

2. Materials and methods

The biological material, supplied by Suceava Agricultural Research and Development Station, was represented by six different seed samples of wheat (*Triticum aestivum* L. spp. *aestivum*) belonging to a local cultivar. Except the sample W1, whose seeds were harvested in 2013, the other ones have included seeds from the same cultivar, but with different storage periods in uncontrolled conditions, as follows: W2 (4 years), W3 (3 years), W4 (9 years), W5 (13 years), and W6 (10 years).

For viability (germination %) assessment there were used 100 seeds for each sample. The distilled water, which imbibed a special filter paper put into Petri boxes, represented the germination medium. In the first stage, the seeds were soaked in water (t = 15 - 17°C) for 4 hours, and water was changed 3 times in the first 24 hours, after each change seeds being left without water for 2 h. In the next 24 hours the temperature was maintained at 16°C, and further at 20°C by the end of the trial (7 days). There were considered germinated the seeds whose rootlets were at least 1 mm length [5].

To determine Total Phenolic Contents (TPC) in wheat samples, first it was obtained an extract for each seeds sample, weighing each 1 g of grain, which were finely ground and subjected to extraction with a mixture methanol and water (80/20), by stirring, centrifuging and recovering the supernatant [3]. The estimation of total phenolic contents in seed extracts was carried out through a colorimetric assay, by measuring its reducing capacity with Folin-Ciocalteu reagent. TPC was expressed as mg Gallic Acid Equivalent/g seed (GAE/g seed). For this purpose, it has generated a standard curve (Fig.1) representing the absorbance values of gallic acid standard solutions in relation to their concentrations [6].

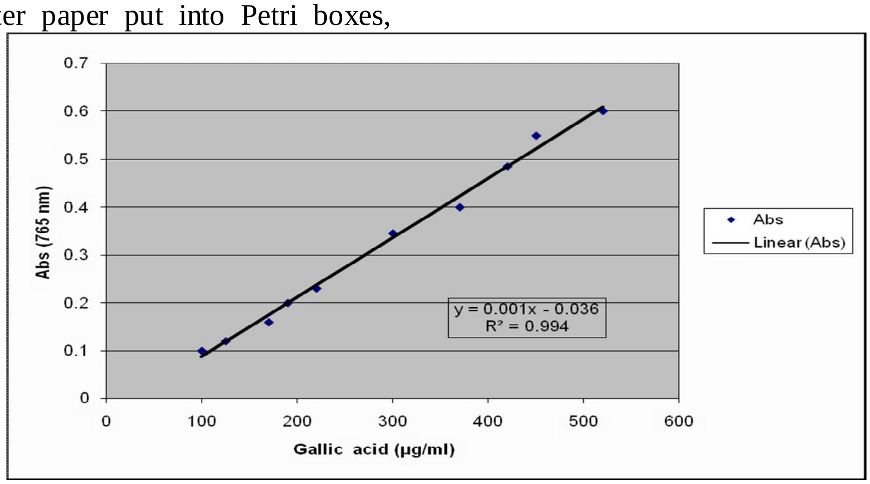

Fig. 1. Standard curve for TPC using gallic acid

The seed exudate electrical conductivity was evaluated using a conductometer supplied by Hanna Instruments. Each sample, containing 30 seeds, has been introduced into Berzelius tumbler with 50 ml distilled water and incubated at 25°C (in the dark). After 6 hours, the distilled water, containing the seed exudate, was collected for measuring its conductivity. The results have been reported as µS/g (µohm/g), seeds being previously weighed [7].

The total sugars dosing in exsudate has been accomplished of 1 g seeds, which were incubated in 10 ml distilled water at 25°C (in the dark). After 6 hours there were dosed the total sugars, using a method based on reducing of picric acid to picramic acid by monoglucides [8, 9]**.** The results were expressed as µg sugars at 1g of seed.

Each determination was made in four replicates, and their data were statistically processed. In order to calculate differences between results, it was used the analysis of variance, the differences at $p < 0.05$ being considered significant.

3. Results and discussion

In the Table 1 are rendered the mean values of viability and Total Phenolic Content (TPC) in six wheat samples.

Table 1

Comparative mean values of viability and TPC in wheat seed samples

Wheat samples	Viability (G%)	TPC (mg GAE/g seed)	
		Before germination	Post germination
W1	96±1	1.38±0.29	2.24±0.08
W2	85±1	1.42±0.18	2.32±0.20
W3	74±2	0.94±0.06	1.72±0.12
W4	66±3	1.16±0.21	1.46±0.07
W5	58±1	0.82±0.03	0.92±0.02
W6	37±2	0.65±0.05	0.70±0.09

Note:
G = germination percentage; TPC = Total Phenolic Content

As seen in the table 1, the highest Viability (G%) has recorded the sample W1, whose seeds were harvested in 2014, and the lowest one the sample W6, with seeds stored for 10 years in uncontrolled conditions. Small Viabilities were also recorded the samples W4 and W5, kept in uncontrolled stored conditions for 9, and 13 years. As to the mean values of TPC before germination, these ones ranged between 0.65 ± 0.05 mg GAE/g (W6) and 1.42 ± 0.18 mg GAE /g (W2). It is found that in seed samples with lower Viability (W3-W6) TPC values (determined before germination) were smaller, with percentages between 18.9 - 22.4 (W4) and between 112.3 - 118.4 (W6), compared to the samples W1 and W2 ($p < 0.05$).

The determination of TPC in germinated seeds has shown values between 0.70 ± 0.09 (W6) and 2.32 ± 0.20 (W2). In post germination also, in seed samples with less germination capacity (W3-W6) TPC values were lower, with percentages between 30.2-34.8% (W3), and between 220 - 231.4% (W6), as compared to samples W1 and W 2 ($p < 0.05$).

Comparing TPC values before and after germination, indicates that the largest percentage increases were recorded, in order, to samples W3 (82.9%), W2 (63.4%) and W1 (62.3%), and the lowest ones in samples W6 (7.7%) and W5 (12.2%).

Searching the antioxidant activities of winter cereal crops before and after germination, Hyun Young Kim et al. (2013) have found a significant increasing (30,6%) of the total phenolic content in wheat seeds from 1.57 mg/g GAE (pre-germination) to 2.05 mg/g GAE (post-germination).

The phenolic content increased significantly during wheat germination for up to 7 days [10]**.** According to Li, Shewry and Ward (2008), the phenolic acids are found in cereal grains in free, soluble conjugate and insoluble bound forms.

The increase in phenolic compounds in the post-germination winter cereals can be explained as an increase in the amount of free forms from hydrolysis due to the dismantling of the cell wall during germination [4].

It is possible that lower values of TPC in seed samples with lower viability to have as cause the reduced activity of some enzymes (eg. hydrolases), which release phenolic acids from conjugated soluble forms, as a result of transformations occurring by longer and in bad conditions seeds keeping.

The correlations between Viability (G%) and TPC, determined before germination (TPCBG), and between viability and TPC, determined post germination (TPCPG) have shown positive correlations (Fig. 2-3), with R^2 = 0.8207 (for the relationship V–TPCBG) and R^2 = 0.91 (for the relationship V–TPCPG).

The Table 2 reproduces the mean values of viability and exudate indices in wheat seeds.

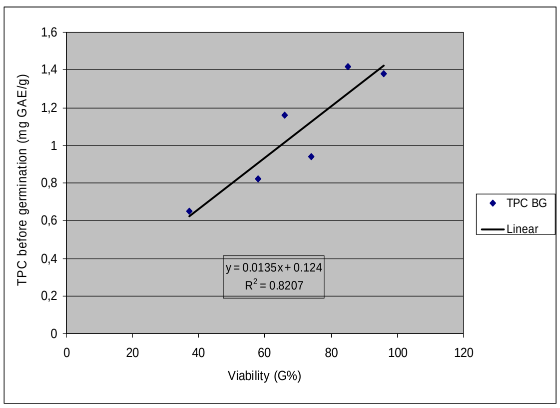

Fig. 2. Linear regression for the correlation between Viability (G%) and TPCBG (mg/g seed)

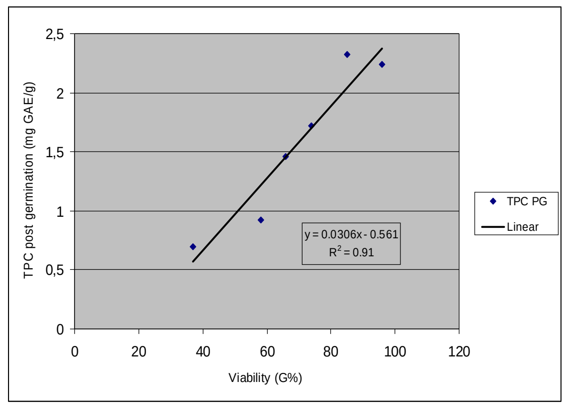

Fig. 3. Linear regression for the correlation between Viability (G%) and TPCPG (mg/g seed)

Table 2

Comparative values of viability, conductivity and sugars efflux in wheat seeds

Wheat samples	Viability (G%)	Conductivity (μS/g)	Sugars Efflux (μg/g)
W1	96±1	24.13±1.2	152.50±5.2
W2	85±1	29.87±0.9	237.35±3.8
W3	74±2	23.20±3.2	270.28±2.9
W4	66±3	28.45±1.5	298.65±11.3
W5	58±1	28.10±0.7	325.16±7.4
W6	37±2	47.35±2.8	462.70±9.7
Note: G = germination percentage			

As can be seen from the table, the mean values of the seeds conductivity were between 23.20 ± 1.2 μS/g (W3) and 47.35 ± 2.8 μS/g (W6). A low value was recorded by W1 (24.13 ± 1.2 μS/g), while samples W2, W4 and W5 showed close values, ranging between 28 and 30 μS/g.

The transmembrane efflux sugars had average values between 152.50 ± 5.2 μg/g (W1) and 462.70 ± 9.7 μg/g (W6).

Compared to conductivity values, which have fluctuated, but have had the highest value in the seeds sample with the lowest Viability, the total content of sugars (sugars efflux) in the seeds exudate has steadily registered values increasing once with decreasing seed viability ($p < 0.05$).

The correlations between Viability (G%) and Conductivity (C), and between Viability and transmembrane Sugars Efflux (SE) in the six wheat seed samples

(Fig. 4-5), have indicated negative correlations, with R^2 = 0.9754 (for the relationship V–SE) and R^2 = 0.6262 (for the relationship V–C).

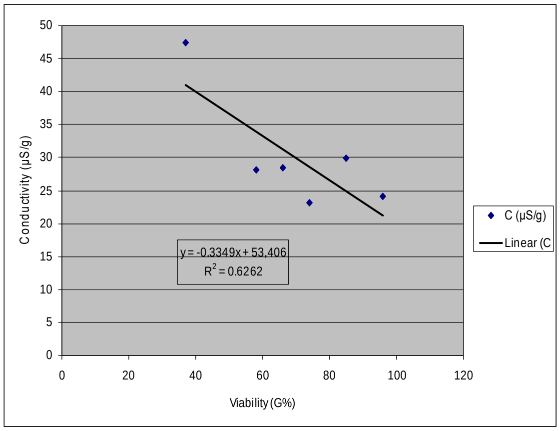

Fig. 4. Linear regression for the correlation between Viability (G%) and Conductivity (µS/g seed)

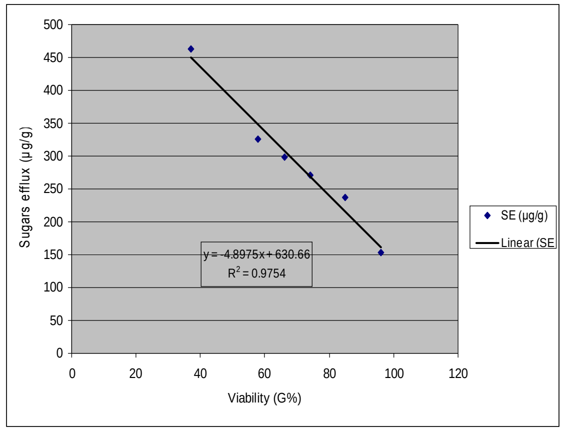

Fig. 5. Linear regression for the correlation between Viability (G%) and Sugars efflux (µg/g seed)

Using the TPC values, determined before seed germination (TPCBG), the conductivity (C), and the transmembrane efflux of sugars (SE) there were calculated the correlations between these indices, which were negative, with R^2 = 0.336 (for the relationship TPCBG–C) and R^2 = 0.7532 (for the relationship TPCBG–SE).

4. Conclusions

Analysing six wheat seed samples (belonging to a local cultivar), with various storage periods, as to relationships between Viability (Germination %), Total Phenolic Content (TPC), conductivity and sugars efflux, there were found different values of these indices as well as negative or positive correlations, more or less significant, depending on seeds viability.
Both before and post germination, the seed samples with higher viability have recorded higher total phenolic content values, compared to samples with low viability.
The germination process has increased the total phenolic content values with much higher percentages in samples with risen viability.

The correlations between viability and total phenolic content determined before and post germination have shown positive correlations significant for relationship Viability – TPC post germination.
Regarding the seminal exudate, the conductivity values have fluctuated, the highest value being in seed sample with lowest viability. The sugars efflux has steadily registered increasing values once with seed viability decreasing.
The correlation between viability and conductivity, as well as between viability and transmembrane sugars efflux in the six wheat samples, has shown negative correlations, significant for relationship viability – sugars efflux.

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