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Original article

Study on the Effect of Cold Plasma on the Germination and Growth of Durum Wheat Seeds Contaminated with *Fusarium Graminearum*

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Abstract

Cold plasmais a potentially new method of controlling diseases caused by fungal pathogens. In this investigation the effect of treatment with cold plasma of durum wheat seeds contaminated with *Fusarium graminearum* on the germination and growth of plants was studied. Plants of 6 durum wheat varieties were pre-contaminated with spore suspension of *Fusarium graminearum*. The harvested seeds were treated with cold plasma in 4 variants: 1– direct treatment with Argon plasma torch sustained by travelling electromagnetic wave; 2 – treatment with the same plasma torch of seeds in 20 ml distilled water; 3 – underwater diaphragm discharge treatment in the container with applied voltage of 15 kV electrode, denoted by "+"; 4 – underwater diaphragm discharge treatment in the container with grounded electrode, denoted by "–". Two control variants were used – dry not treated contaminated seeds and wet not treated contaminated seeds. After the treatment the seeds were placed in petri dishes for germination. Sprouted seeds were planted in pots with soil mixture and cultivated to maturity in green house conditions in Field Crops Institute –Chirpan, Bulgaria in 2017/2018 year. The effect of the treatment on the following traits were studied: germination rate, days to heading, plant high, parameters of chlorophyll fluorescence during the grain filling, spike length, kernel number per spike, kernel weight per spike, TKW and obtained ill (*Fusarium graminearum*) and healthy seeds. The results received were processed statistically via two-way ANOVA and Duncan's multiple range test. The analysis of variance reveals that the genotype, treatment with cold plasma and the interactions between them have a statistically significant effect on the variation of the germination rate. The best germination rate (means from all genotypes) was obtained by treatment with cold plasma variant 1 - direct treatment with Argon plasma torch sustained by travelling electromagnetic wave and variant 3 - underwater diaphragm discharge treatment in the container with applied voltage of 15 kV electrode, denoted by "+". After the germination the number of seeds contaminated with *Fusarium graminearum* was the lowest after variant 4 in the treatment of three of the studied varieties. Stimulating effect of the cold plasma treatment on the plant growth was found in 4 genotypes. Varieties Elbrus, Progres, Deni and Zvezdica were with higher PH during the grain filling. The results from the influence of cold plasma on the other studied traits will be processed after the plant maturation and will be included in the final version of the paper.

Keywords: Cold Plasma, Fusarium Contamination, Durum Wheat.

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INTRODUCTION

Unbalanced low pressure plasma discharge has been widely used for decades in a number of technologies such as the production of: microelectronics chips, polymers, and energy-saving gas discharge lamps. In the last decade, intensive work has been done on plasma applications in areas distant from physics and engineering such as medicine, biology, and agriculture. The main constraint for expanding technological applications of low-pressure plasma is the need for a complex and costly vacuum system in which samples are treated. When working with biological systems and living organisms, treatment in a vacuum chamber is virtually impossible. Therefore, in recent years, new plasma sources working at atmospheric pressure have been intensively developed. In some of the medical applications of plasma at atmospheric pressure mainly heat effects (high plasma gas temperature) for sterilization, argon plasma coagulation and plasma scalpel for tissue removal are used. However, in many cases treatment should be carried out without thermal damage, while ensuring high bactericidal effect. The basic requirement when working with biological objects is plasma to be unbalanced and cold, with an average energy of electrons ~ 1-2 eV, while the temperature of the heavy particles is significantly lower and close to room temperature. The creation of "cold atmospheric plasma" (CAP) allows its application for treating living tissues, thermo-sensitive materials, foods, seeds, etc. Most commonly, for obtaining "cold atmospheric plasma" the following are used: Dielectric Barrier Discharge (DBD) operating at different frequencies and a microwave plasma torch, in which the discharge is created and maintained by a travelling electromagnetic wave. The microwave plasma torch has a number of advantages, such as the absence of electrodes and correspondingly high plasma purity, a small and compact wave exciter, good stability and reproducibility of the plasma characteristics. Until now, it has not been used for biomedical and agronomic applications since the gas plasma temperature is usually too high 1000-2000 K. At present, PlasmaLab laboratory at Sofia University, Bulgaria, has achieved near-room gas plasma temperature in the area of action on biological objects.

The number of potential applications of cold plasma in agriculture has grown significantly in recent years. It is considered that cold plasma treatment is a fast, economic and pollution-free method to improve seed performance, plant growth and ultimately plant production (Dhayal, 2006; Tong et al., 2014). It has been found that this treatment plays a crucial role in a broad spectrum of plant development and physiological processes, including the promotion of seed germination and seedling growth (Šerá, 2008; Li et al., 2014), activation of photosynthesis (Šerá et al., 2010; Chen et al., 2012), regulation of carbon and nitrogen metabolism (Zhou, 2011; Henselová et al., 2012). There are reports about positive influence of cold plasma on the tolerance of plants to biotic and abiotic stress factors as disease and drought. (Jiang et al., 2014; Li et al., 2015) .

Although cold plasma is widely used in medicine for the inactivation of microorganisms and decontamination of instruments (Iseki et al., 2010), there are still limited studies demonstrating its potential for impact and control of fungal pathogens in economically important plant species (Selcuk et al., 2008; Iseki et al., 2010; Filatova et al., 2011; Nishioka et al., 2013; Nishioka et al., 2014). According to Kordaset et al. (2015), the treatment of winter wheat seeds with cold plasma leads to a reduction in the number of fungal colonies developing on seeds, and according to (Nishioka et al., 2013), cold plasma is effective even with regard to the inactivation of seed transferable diseases.

The Fusariosis disease on spikes can be of great economic importance to cereal crops in cold and humid weather conditions. The harmful effect of the disease is also enhanced by the intense synthesis of toxins, which make the attacked grain unfit for consumption by both humans and animals (Stancheva, 2002). The cause of the disease are fungi of the genus Fusarium. The control of fusariosis on the spikes is very difficult due to the wide specialization of the causative agents, the lack of resistance and the mass distribution of the pathogen in nature. In recent years the development of alternative approaches for control of seed-borne fungal pathogens, incl. fusarium in durum wheat is a serious challenge for plant phytopathologists.

In this investigation the effect of treatment with cold plasma (microwave plasma torch) on the germination, % of infected seeds, growth and productivity of plants of seeds of 6 durum wheat varieties artificially infected with *Fusarium graminearum* was studied.

Material and Methods

Plants of 6 Bulgarian durum wheat varieties – Elbrus, Victoria, Progres, Predel, Deni and Zvezdica were artificially contaminated with spore suspension of Fusarium graminearum in field trial, conducted in Field Crops Institute – Chirpan, Bulgaria during 2016/2017 growing season. Pre-labeled and prepared spikes were sprayed with a spore suspension of 14-day grown culture of the pathogen. The seeds obtained from the infected spikes were collected in a complete mature phase. The collected seeds were treated with cold plasma (microwave plasma torch) before sowing in 4 variants: direct treatment with argon plasma torch sustained by travelling electromagnetic wave (Var. 5); treatment with the same plasma torch of seeds, immersed in 20 ml distilled water (Var. 6); underwater diaphragm discharge treatment in the container with the applied voltage of 15 kV electrode, denoted by "+" (Var. 3) and underwater diaphragm discharge treatment in the container with the grounded electrode, denoted by "– " (Var. 4). Two control variants were used, too - dry not treated contaminated seeds (Var. 1) and wet not treated contaminated seeds (Var. 2).

The plasma source was constructed in the PlasmaLab of Sofia university, Bulgaria and consisted of microwave generator coupled to a surfatron-type waveguide producing a travelling electromagnetic wave that propagates along a quartz tube. The discharge is created in an Ar (99.99999%) flow and the plasma gas temperature does not exceed 40 ^{o}C.

After the treatment the seeds were placed in petri dishes for germination at 24 ^{o}C in a thermostat. Sprouted seeds were planted in pots with soil mixture and cultivated to maturity in green house conditions in Field Crops Institute –Chirpan, Bulgaria in 2017/2018 year. The experiment was performed as randomized block design in three replications for each genotype and each treatment variant. The effect of the cold plasma treatment on the following traits was studied: germination rate, days to heading, plant height, parameters of chlorophyll fluorescence during grain filling, spike length, kernel number per spike, kernel weight per spike and obtained ill (*Fusarium graminearum*) and healthy seeds.

The chlorophyll fluorescence was measured on flag leaves for all variants of treatment with cold plasma in cultivar Elbrus by portable chlorophyll fluorometer - MINI-PAM – walz-gmbh – Germany. All measurements were carried out during the grain filling stage of the durum wheat in the morning up to 11 a.m. The minimal fluorescence yield of the illuminated samples (F) and the maximal fluorescence yield (f'm) of the illuminated light-adapted sample were measured with every saturation pulse.The variable fluorescence yield of the light-adapted leaf was calculated according to (fv=f'm-f). The quantum yield of photochemical energy conversion of PS II was estimated according to: Y = FV/ f'm (Genty et al., 1989)

The results obtained were processed statistically via two-way ANOVA and Duncan's multiple range test through TIBCO statistica 13.3.0 software package.

Results and discussions

Different variants of cold plasma treatment have different effect on the germination of fusarium-infested seeds in the studied durum wheat varieties (Table 1)

Table 1. Germination rate of durum wheat genotypes treated with cold plasma.

Genotype	Germination rate %						
	Variants of treatment with cold plasma						
	Var.3	Var.4	Var.5	Var.6	Var. 1	Var.2	Average
Elbrus	93.3	68.3	78.3	76.7	44.0	50.0	68.4
Victoria	92.7	74.7	80.3	65.0	96.7	82.3	81.9
Progres	86.7	91.6	93.3	73.3	93.3	95.0	88.8
Predel	100.0	91.7	91.0	88.3	90.0	75.0	89.3
Deni	78.7	63.0	93.3	86.0	93.3	86.7	83.4
Zvezdica	77.6	69.3	96.7	83.3	96.0	89.3	85.3
Average	88.2	76.4	88.8	78.8	85.5	79.7	

The conducted variance analysis shows that the genotype, cold plasma treatment and the interaction between genotype and treatment have statistically significant influence on the observed variation in the percentage of germinated seeds (Table 1).

In the underwater diaphragm discharge treatment Var. 3 the percentage of germinated seed (average of all genotypes) was 88.2%, which exceeded by 8.5% the germinated seeds of the control variant - 79.7 (Var.2 - wet not treated with CP contaminated seeds). This treatment option increased germination in 4 of the tested varieties: Elbrus - by 43.3%, Predel - by 25.0%, Zvezdica - by 11.7%, Victoria - 10.4% and decreased it in the Progres and Deni cultivars. In direct treatment with argon plasma torch sustained by travelling electromagnetic wave (Var. 5) the germination rate on average of all genotypes also increased, but negligibly compared to the control variant Var. 1.

Furthermore in both varieties Elbrus and Predel with the highest increase in germinationation rate was recorded the lowest percentage of contaminated seeds of Fusarium gramiearum (counted during seed germination) at all cold plasma exposure variants in comparison to the control variants. In cultivars Zvezdica and Deni at diaphragm discharge treatment Var. 3 was recorded the lowest percentage of contaminated seeds in comparison with the control variant (Var.2). In the other two cultivars there was an increase in the infected seed after all variations of cold plasma treatment. The conducted variance analysis shows that genotype, cold plasma treatment and the interaction between genotype and treatment have a statistically significant influence on the observed variation in the number of infected seeds (Table 2).

Table 2. Analysis of variance of the studied traits

Analysis of variance of the studied traits Traits	Source of variation and % of total variation					
	Genotype (G) df -5		CP Treatment (T) df -5		Interaction (G×T) df -25	
	MS	η^2, %	MS	η^2, %	MS	η^2, %
Germination rate	**1057.3*** **	**29.12**	**499.5** **	**13.76**	**414.6** **	**57.11**
Plant Height	**317.7** **	**35.95**	**229.3**	25.94	**67.3***	38.2
Spike length	**13.44** **	**88.7**	**0.289**	1.90	**0.283**	9.29
Kernel number in spike	**402.44** **	**46.44**	**133.56***	**15.4**	**66.12**	38.14
Kernel weight per spike	**1.51** **	**54.55**	**0.115**	3.05	**0.2283**	41.24

*** Significant at p< 0.001; ** Significant at p<0.01; * Significant at p<0.05

The interaction between genotype and treatment has the greatest impact on the observed variation of this trait - 53.7% of the total variation, and supports the established varied reactions of cultivars to cold plasma treatment.

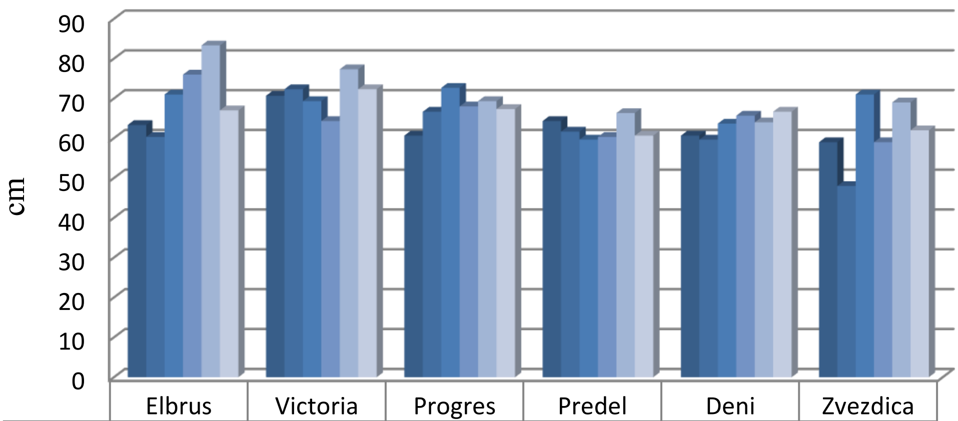
On the basis of the results obtained, the following conclusions can be drawn: the underwater diaphragm discharge treatment Var. 3 increases the germination rate in 4 of the studied genotypes by 22.6 % in comparison with not treated seeds. In two of all studied genotypes a decrease in the number of infected seeds in all cold plasma exposure variants was observed in comparison to not treated seeds.

Our results correlate to the studies by Jiang et al. (2014) in wheat and Li et al. (2014) in soybean, who found that treatment with cold helium plasma of non-infected seeds can increase the germination rate but the effect is different depending on the output power of used cold plasma. Increasing the germination rate of fusarium-contaminated seeds is an important step in controlling the consequences of infection because pathogens causing the disease cause a significant reduction or complete loss of germination ability. In literature there is a limited number of reports demonstrating the possibilities of cold plasma treatment for the inactivation of seed-transmissible pathogens in seeds (Selcuk et al., 2008; Nishioka et al., 2013; Nishioka et al., 2014). Nishioka et al. (2014) reported a drastic reduction in the survival rate of the R.solani pathogen in cold plasma treated brassicaceous seeds. Our results, showing a significant reduction in % of the Fusarium-infected durum wheat seeds in two of the cultivars tested in all treatments used, support the possibility of inactivation of seed-transmissible pathogens by exposure to cold plasma. However, research needs to be deepened by conducting an additional phytopathological pathogen survival test.

After the conducting of the germination test the sprouted seeds of all genotypes and treatment were planted in pots and cultivated to maturity in green house conditions. The following traits related to growth and productivity were observed: plant height (PH), spike length (SL), parameters of chlorophyll fluorescence during the grain filling, kernel number per spike, kernel weight per spike and obtained ill (*Fusarium graminearum*) and healthy seeds after the harvest.

The conducted analysis of variance for 6 genotypes and 6 variants of treatment with cold plasma reveals that the effect of genotype (G) on the variation of all studied traits related to growth and productivity was significant (Table 1). The effect of treatment with cold plasma is significant only for kernel number per spike. The interaction between genotype and treatment with cold plasma is significant for plant height.

Plant height of treated with Var. 5 seeds (means of all genotypes) was 8.44 cm higher than that of the control variant 1. (Fig.1).

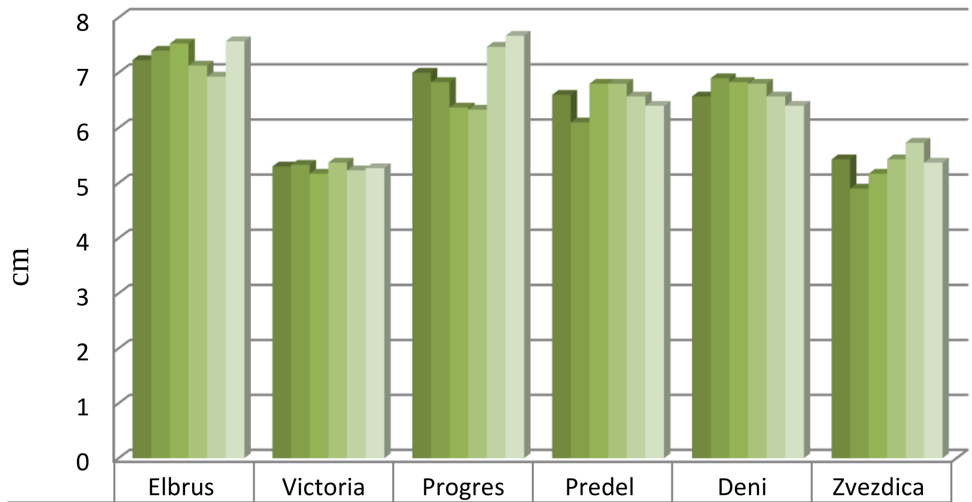


	Elbrus	Victoria	Progres	Predel	Deni	Zvezdica
Var. 1	63,33	70,67	60,67	64,33	60,67	59
Var. 2	60,33	72,33	66,67	61,67	59,67	48
Var. 3	71	69,33	72,67	59,67	63,67	71
Var. 4	76	64,33	68	60,33	65,67	59
Var.5	83,33	77,33	69,33	66,33	64	69
Var. 6	67	72,33	67,33	60,67	66,67	62

Figure 1. Influence of CPT on plant height of durum wheat cultivars

The plants originating from treated with Var. 5 seeds at all studied cultivars have a longer stem than the control plants, with the greatest increase reported for the cultivars Elbrus – 20 cm and Zvezdica – 10 cm. Treatment variants 3, 4 and 6 have also resulted in an increase of plant height in cultivars Elbrus, Progres, Deni and Zvezdica compared to the plants in the control variant (Var. 2), the greatest increase having been observed in Var. 3 for Zvezdica cultivar - 23 cm and in Var. 3 for Elbrus cultivar - 15.7 cm over the control.

Cold plasma treatment did not have a significant effect on the spike length. (Fig. 2).

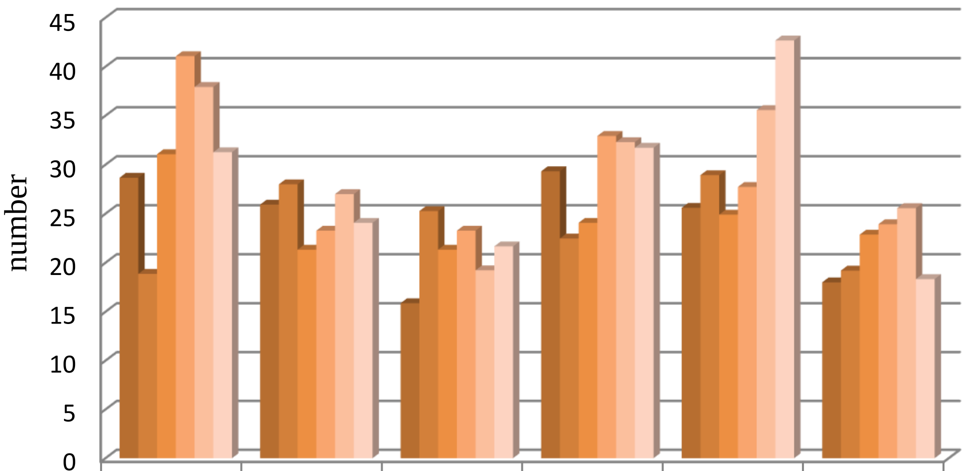


	Elbrus	Victoria	Progres	Predel	Deni	Zvezdica
Var. 1	7,23	5,3	7	6,6	6,57	5,43
Var. 2	7,4	5,33	6,83	6,1	6,9	4,9
Var. 3	7,53	5,17	6,37	6,8	6,83	5,17
Var.4	7,13	5,37	6,33	6,8	6,8	5,43
Var. 5	6,93	5,23	7,47	6,57	6,57	5,73
Var. 6	7,57	5,27	7,67	6,4	6,4	5,37

Figure 2. Influence of CPT on spike length of durum wheat cultivars

A weak and statistically not significant increase of this trait has been observed in the 6th treatment variant (Var. 6) for cultivars Elbrus, Progres, Predel and Zvezdica. and in the 5th treatment variant for cultivars Progres and Zvezdica. Cultivar Victoria did not respond to any of the treatment variants and the spike length of the plants from the treated variants was almost the same as the control plants.

The number of kernels in the spike and the weight of kernels in the spike are important traits that determine to a great extent the plant productivity. The variance analysis shows that the cold plasma treatment of seeds has a statistically significant effect on the variation in grain number in the spike and is responsible for about 16% of the total variation. The interaction between genotype and treatment, although statistically unproven, accounts for 38 % per cent of the variation in this trait.



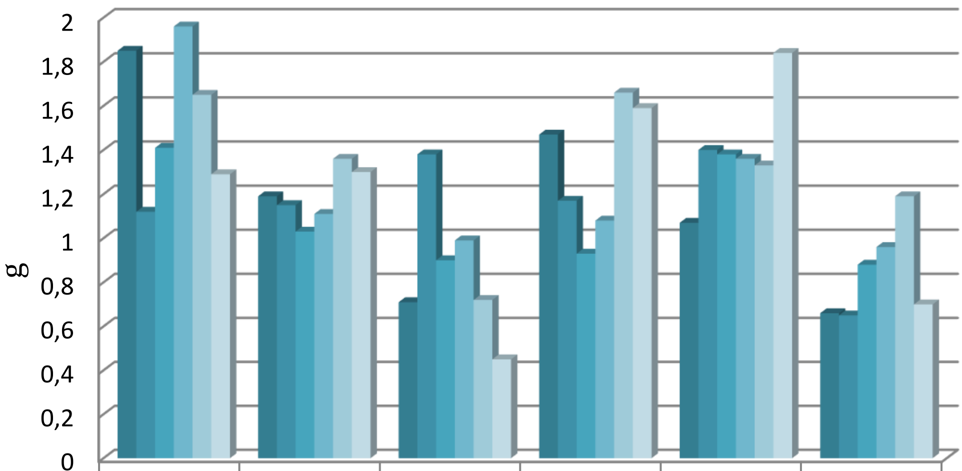
	Elbrus	Victoria	Progres	Predel	Deni	Zvezdica
Var. 1	28,67	25,93	15,87	29,33	25,6	18
Var. 2	18,87	28	25,27	22,47	28,93	19,2
Var. 3	31,07	21,33	21,33	24,07	24,9	22,87
Var. 4	41,07	23,27	23,27	32,93	27,73	23,93
Var.5	37,93	27	19,23	32,3	35,57	25,57
Var.6	31,27	24,07	21,67	31,73	42,67	18,33

Figure 3. Influence of CPT on kernel number per spike of durum wheat cultivars

The number of grains per spike increased in all variants of cold plasma treatment compared to the control plants of Elbrus, Predel and Zvezdica cultivars. In the Elbrus and Predel cultivars the number of kernels per spike increased to the highest extent in the treatment variant Var. 4, with the Elbrus cultivar giving 22.2 pcs. more kernels per spike, and for Predel cultivar - 10.5 pcs. more. In Zvezdica cultivar Var. 5 resulted in the greatest increase of this trait compared to the control variant (Var.1).

For Deni cultivar a greater number of kernels per spike were obtained in only two of the treatment variants Var. 5 and Var.6. For the cultivars Victoria and Progres the number of kernels is increased only in Var. 5, and in all other treatment variants a smaller number of kernels per spike was obtained compared to the control Var.2. Treatment Var. 5 had a positive effect on this trait in all tested cultivars with an average of 5 more kernels being obtained from all reacting cultivars than the control variant (Var.1). The largest increase in the number of kernels in this variant was obtained in the cultivars Deni - 9.97 pcs. more than the control and in the Elbrus cultivar - 9.26 pcs. more.

The kernel weight per spike is increased to the greatest extent in treatment variant Var. 5 an average of 26.2 % in 5 of the cultivars compared to the control (Var. 2) (Fig. 4).



	Elbrus	Victoria	Progres	Predel	Deni	Zvezdica
Var. 1	1,85	1,19	0,71	1,47	1,07	0,66
Var.2	1,12	1,15	1,38	1,17	1,4	0,65
Var.3	1,41	1,03	0,9	0,93	1,38	0,88
Var.4	1,96	1,11	0,99	1,08	1,36	0,96
Var.5	1,65	1,36	0,72	1,66	1,33	1,19
Var.6	1,29	1,3	0,45	1,59	1,84	0,7

Figure 4. Influence of CPT on kernel weight per spike of durum wheat cultivars

The increase varies significantly among the different cultivars – from 1 % in cultivar Progres to 80 % in cultivar Zvezdica. Treatment variant 6 Var.6 increases kernel weight per spike also in 5 cultivars varying from 7 % in cultivar Zvezdica to 36 % in cultivar Predel. Cultivar Zvezdica has had a positive reaction to all cold plasma treatment variants, Var. 3, Var. 4 also having increased kernel weight significantly – by 35 % and 47 %, respectively. Cultivar Progres is the only cultivar that had not responded by increase in kernel weight per spike in cold plasma treatment.

Chlorophyll fluorescence is used for estimation of the functional state of photosynthetic apparatus. The quantum yield (Y) of plants in cultivar Elbrus in all variants of treatment with cold plasma is higher than both used control variants and ranged between 0, 51 for the control (Var.1) to 0.58 for Var.5 and between 0.39 for control 2 (Var. 2) to 0.57 for Var.6, (Fig. 5).

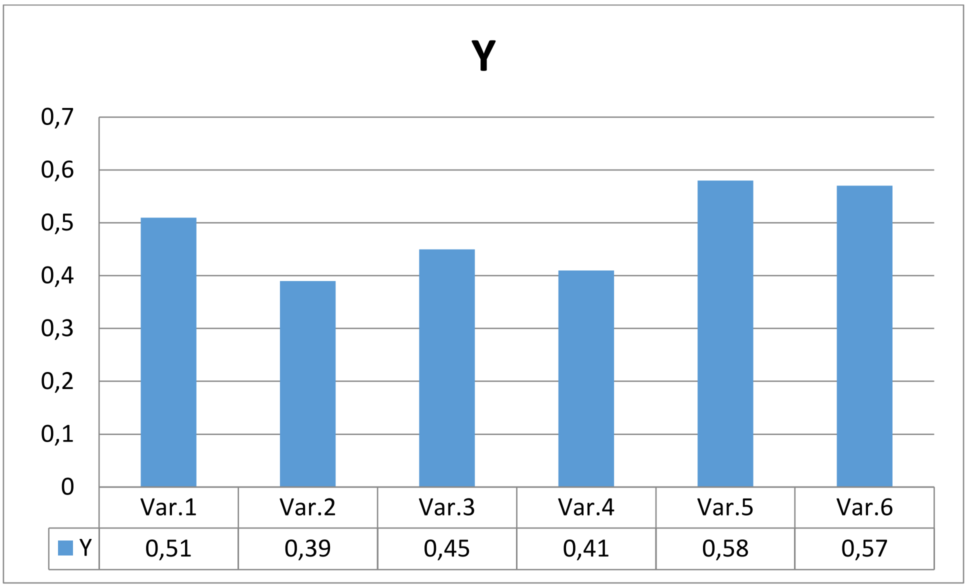


Figure 5. Influence of CPT on QY of photochemical energy conversion of PS II at cultivar Elbrus

The found increase in the quantum yield (Y) in plants of cultivar Elbrus treated with cold plasma reveals the possibility of CPT for enhancing the capacity for absorption and use of light and thus for increase of the efficiency of photosynthesis in plants, originating from contaminated with *Fusarium graminearum* seeds. Saberi et al. (2018) also found improvement in the intensity of photosynthesis in wheat following treatment with Nonthermal Radio Frequency Plasma

The results from this study show that cold plasma treatment of seeds can increase not only the germination rate of Fusarium-infected seeds, but also improve plant growth and productivity for most of the studied durum wheat cultivars. In recent years the number of publications has increased reporting a positive impact of cold plasma on the growth and productivity of plants of commercially important species in later stages of development and during ripening. Filatova et al. (2011) established that plasma seeds treatment owing to its stimulatory and fungicidal effects increased crop yield of spring wheat by 4-6%, maize – by 1.5-2.0%, lupine – by 20-40% as compared to control.

Conclusions

The conducted analysis of variance for 6 genotypes and 6 variants of treatment reveals that the effect of cold plasma treatment is significant for germination rate, number of contaminated seeds during the germination and kernel number per spike. The interaction between genotype and treatment with cold plasma is significant for germination rate, number of contaminated seeds during the germination and plant height.

The treatment of artificially infected with *Fusarium graminearum* seeds with cold plasma Var.3 (underwater diaphragm discharge treatment in the container with the applied voltage of 15 kV electrodes, denoted by "+") has a positive impact on the seed germination at 4 of the studied genotypes.

The used durum wheat varieties respond differently to the applied cold plasma exposures, expressed as a percentage of germinated seeds.

In two of all studied genotypes a decrease in the number of infected seeds was observed in all cold plasma exposure variants in comparison to not treated seeds.

Var. 5 had the highest positive effect on the studied traits related to plant growth and productivity: plant height, spike length, number of grains per spike and grain weight per spike in plants of 6 durum wheat varieties, originating from artificially contaminated seeds.

Cultivars Victoria and Progres had reacted to the least extent to cold plasma treatment.

The found increase in the quantum yield (Y) in cold plasma treated plants of cultivar Elbrus reveals the possibility of CPT for enhance of the capacity for absorption and use of light and thus for increasing the efficiency of photosynthesis in plants.

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