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EFFECT OF ULTRASOND TREATMENT ON THE SEEDS OF DIFFERENT GLADIOLUS (*GLADIOLUS HYBRIDUS* L.) VARIETIES ON THE SOWING QUALITY

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Abstract

The main aim of the present study was to investigate the possibility of the application of ultrasound for stimulating the viability behaviors of gladiolus seeds. The studies were carried out with seeds of three varieties of gladiolus. Seeds were treated with ultrasound for 2, 4, 6, and 8 min. Germination energy, germination, main germination time, uniformity of germination, time of 50% germinated seeds, seedling morphology- length of embryo root, length of hypocotyls and fresh weight were reported. The correlation dependences on the seedling's weight with the length of embryo root and hypocotyls, as well as a linear polynomial regression between the treatment period and germination, characterized with high determination coefficients were established. The highest viability was reported in the treatment of 6 minutes of ultrasound, approximately 20% above the control. These results can be used in hybridization breeding to accelerate seed development in the propagation of corms.

Key words*: germination, gladiolus, stimulation, seedling morphology, viability.*

INTRODUCTION

Gladiolus is one of the main ornamental crops, which ranks fourth in importance in world trade (Farhat T., 2004). The genus *Gladiolus* belongs to the family *Iridaceae* (Raj et al., 2009; Azim et al., 2019) and according to some authors, such as Goldblatt. & Manning, (1998), about 255 species are currently known in this genus. The plant is native to the region of southern and tropical Africa, Madagascar and Eurasia (Takatsu et al., 2001). The species is grown mainly for cut flowers and less often for landscaping (Manning and Peter, 2008; Memon et al., 2013). Its valuable decorative properties are related to the beautiful flowers, a wide diversity of colours and upright flower-bearing stems, as well as the wonderful aroma and long vase life (Azim, 2019).

Most modern cultivars of *Gladiolus x grandiflora* are the result of selection and hybridization between six species (Barnard, 1972). At the moment, the main research activity is aimed at creating new varieties with higher decorative qualities, more diverse forms and colouring of flowers. In modern breeding, hybridization, both between different cultivars and interspecies, is a major method and tool to solve these tasks and to increase the variety of forms and cultivars (Hort et al., 2012). Gladiolus cultivars used are complex hybrids between different species (Huxley et al., 1992). After carrying out the crosses in the hybridization, the selection work continues with obtaining seeds and reproduction of the offspring from them. However, the production of high-yielding hybrid gladiolus seeds and their utilization, that is important about hybrid selection, is not always an easy task and successful (Takatsu et al., 2005). Production of gladiolus plants by seed is a relatively long period spanning several growing seasons (Bose et al., 2003; Ramzan et al., 2010).

The obtained seeds during hybridization are a relatively limited number, not always with high germination. Therefore, current science is directed toward researching various methods and ways to improve their vitality and reduce germination time (Basra et al., 2003; Ramzan et al., 2010). In this regard, Carpenter et al. (1991) reported that it is best to store the obtained hybrid seeds of gladiolus, intending to maintain good sowing qualities, due to the relatively long period between seed collection and sowing, at a temperature in the range of 5 to 15°C. Improvement of germination processes of gladiolus seeds Ramzan et al. (2010) achieved by pre-treatment of the gladiolus

seeds with potassium nitrate (KNO_3), reaching a germination rate of 92%. Good results in the treatment of gladiolus seeds, also with potassium nitrate, were also reported by Memon et al. (2013). In evaluating gladiolus seed priming Mushtaq et al. (2012) established a significant improvement in germination.

There are relatively few scientific reports on the application of different physical methods of impact to improve the vitality and the sowing qualities of gladiolus seeds. Carpenter et al. (1991) studied the effect of temperature and air humidity on the gladiolus seeds viability status. There is almost no research on the effect of ultrasound treatment on seeds of this species. Aladjadjiyan (2007) emphasized that the application of ultrasound is an extremely promising and effective way to improve the vital status of seeds.

The main goal of the present study is to investigate the influence of ultrasound treatment on the vitality behaviours of gladiolus seeds, with the aim of accelerating their development.

MATERIALS AND METHODS

The experiments were carried out in the Experimental field and Scientific laboratory of the Department of Horticulture at the Agricultural University - Plovdiv, Bulgaria. Seeds of the following gladiolus cultivars Purple flora, Oscar, and Plum tart, were produced by growing the plants through corms. After flowering and fruit formation, in at full botanical maturity stage, the fruits were harvested, the seeds were extracted and they have been stored under laboratory conditions in paper pouches. Three months after the collection of the seed, they were treated with ultrasound in the following variants:

1. Control (not treated seeds); 2. Ultrasound -2 min; 3. Ultrasound - 4 min; 4. Ultrasound - 6 min; 5.Ultrasound - 8 min.

The treatment has been completed with an ultrasonic bath Nahita, model 620-1 of the company Auxilab, S.L. from Spain with the following parameters: frequency 40 KHz, volume 0.6 l.

After treatment of the seeds, their vital signs were evaluated. Seeds were placed in 10 cm diameter Petri dishes with Watman 1 filter paper moistened with 5 ml of water. Germination energy and germination were determined, according to the requirements of ISTA (2013) and under the recommendations in the manner described by (Ramzan et al., 2010), as 100 seeds were put in four replicates. The time to germination of 50% of the seeds (T50) was determined according to Farooq (2005). Mean germination time (MGT), described by Kader (2005) and Uniformity of germination (described by Panayotov, 2015) were calculated. When determining germination, the weight of the sprouts was established from all germinated seeds in the 4 repetitions and recalculated for one sprout. At this stage, the embryo root length and hypocotyls length of ten seedlings from the 4 replicates were measured.

Data were subjected to ANOVA analysis of variance, correlation and regression analyses (Fowel and Cohen, 1992).

RESULTS AND DISCUSSIONS

The influence of different methods and ways of pre-sowing impacts on crops is established very well by studying their sowing behaviours. One of the important characteristics in this scope is the germination energy. Its values are established for a shorter period, which gives reason to consider these earlier germinated seeds that are with higher vitality (Panayotov, 2015). The effect of different durations of exposure to ultrasound is very well demonstrated (Table 1). A strong varietal reaction is also observed. Sonication for up to 6 min produced a gradual increase in seed germination compared to the control, except for Plum tart. This tendency is most strongly observed in the Purple flora variety, where the difference with untreated seeds reaches 20%. A similar state is reported for the Oscar variety, but with a lower difference of 12.67%. However, the effect on Plum tart seeds was the strongest at 2 min of sonication, with an increase of 10%, after which it decreased, but the values were higher than those of control seeds. A strong pronounced inhibition in all three varieties is established at the 8-minute variant, with a decrease, compared to the control, between 16.67% (Plum tart) and 23.33% (Purple flora).

Table 1. Sowing parameters of gladiolus seed after application of ultrasound

№	Variants	Germination energy (%)			Germination (%)			Time of sprouting of 50% of seeds (T_{50}) (%)		
		Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart
1	Control	46.66	40.00	46.66	73.33	83.33	70.00	7.1	7.3	6.9
2	2 min	53.33	43.33	56.66	76.66	86.66	73.33	6.7	7.2	5.6
3	4 min	56.66	43.33	50.00	76.66	66.66	83.33	6.4	6.3	5.9
4	6 min	66.66	53.33	50.00	93.33	66.66	90.00	6.3	6.3	6.7
5	8 min	23.33	23.33	26.66	46.66	50.00	53.33	8.2	9.0	8.6
LSD p = 0.05		11.2	6.4	8.1	11.2	14.3	9.1	1.4	1.8	1.0

The vital status of seeds is almost fully determined using germination. The values of this indicator fully reflect the quality of the seeds and are used to standardize their sowing and agronomic value (Black et al., 2006). A very pronounced variety response is also observed for this characteristic. This parameter increases uniformity to sonication at 6 minutes was found in Purple flora and Plum tart, with germination reaching 93.33% and 90.0%, respectively, or 20% more than untreated seeds. For Oscar, the highest values were observed in variant 2 minutes, and the increase compared to the control was significantly smaller - by 3.33%. In this variety, in the remaining two periods of ultrasound application, a reduction compared to the control was observed. A very strong suppressive effect, as for germinating energy, was reported at 8 minutes for all three studied varieties. Compared to the control, the reduction was 16.67% for Plum tart to 33.33% for Oscar. The data are mathematically proven except for germination for the three cultivars and germination energy for Oscar. Aladjadjiyan (2007) points out that ultrasound, representing mechanical waves of high frequency, which in plant organism, including seeds, cause an increase in molecular energy and changes in the structure of substances. According to this author, the sonication of seeds for 5 minutes there is a strong effect and increases the germination energy and germination (Aladjadjiyan, 2007a). Similar conclusions were reported by Miano et al. (2015) and Patero and Augusto (2015). Stimulating influences of ultrasound on the germination of seeds of ornamental and flower crops and its uniform change depending on the duration of exposure were reported also by Panchev (2022). The above-mentioned tendency for changes in germination depending on the duration of exposure to ultrasound is complemented very successfully through the established regression relationships (Figure 1). The type of regression in all three varieties is polynomial with high coefficients of determination $R^2 = 0.55$, $R^2 = 0.89$ and $R^2 = 0.94$, respectively for Purple flora, Oscar and Plum tart. Through these coefficients, is possible to determine how the factorial variable affects the dispersion percentage of the resulting variable. Correspondingly between 55% and 94% of the cases, sonication will produce the indicated changes in the germination of the gladiolus seeds.

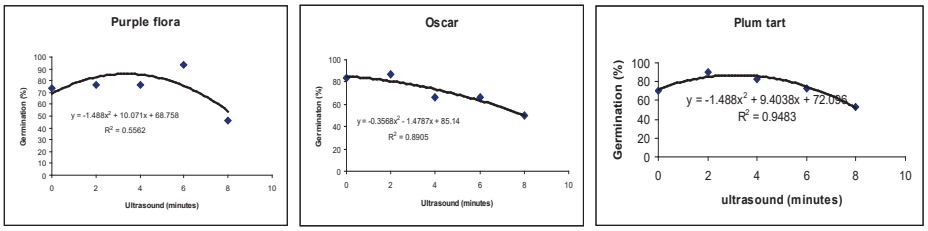

Figure 1. Regression dependence of germination and the duration of ultrasound treatment of gladiolus seeds

The time for germination of 50% (T50) of gladiolus seeds under the influence of ultrasound changes in a narrow range. This time for controlling seeds is approximately 7 days. In the varieties Purple flora and Oscar, it is the shortest at sonication of 6 minutes, and in the first two durations the effect is weaker, but the results are better than those of untreated seeds. Differences from the above-mentioned are observed for Plum tart. The least days for germination of 50% of the seeds of this cultivar were under ultrasound for 2 minutes, after which it increased but was still better than the control. Seed germination was accelerated. As with germination, the highest treatment duration of 8 minutes resulted in a high deterioration, this time increasing approximately between 15% and 25% depending on the variety.

Mean germination time (Table 2) is an essential indicator for evaluating the sowing and viable quality of the seeds and indicates the average number of days that were needed for one seed to germinate (Traynov, 2021). The most as a result of the application of ultrasound for 6 minutes, and depending on the variety, the improvement compared to the control was between 10.7% (Purple flora) and 31.03% (Plum tart). Duration of 2 and 4 minutes also have a stimulating effect on this trait, although weaker. A strong inhibitory effect was found at 8 minutes, with the increase over control being greater by 20.8% to 34.21% for Purple flora and Plum tart, respectively. Uniformity of germination provides significant information about the behaviour of seeds sowing under the influence of a given impact on them. An increase in the uniformity of germination was observed at all tested durations, except for 8 minutes. The best results for the varieties Purple flora and Plum tart were recorded at 6 minutes of ultrasound, and for Oscar - at 4 minutes, approximately 7% above the untreated seeds. The strongest influence was found for Purple flora seeds. As with the other signs discussed above, a pronounced inhibition was present after exposure by 8 minutes, with values decreasing by approximately 30% than to the control.

Table 2. Sowing parameters of gladiolus seeds under the effect of ultrasound application (days)

№	Variant	Mean germination time (days)			Uniformity of germination (%)		
		Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart
1	Control	7.2	7.5	7.6	13.3	11.7	12.4
2	2 min	6.7	7.3	7.0	18.3	16.7	14.4
3	4 min	6.5	7.0	6.3	20.1	18.9	15.1
4	6 min	6.5	6.3	5.8	20.9	16.1	18.9
5	8 min	8.7	9.8	10.2	9.7	8.8	9.3
LSD p =0.05%		1.6	1.0	1.3	3.8	5.2	3.2

Treatment of the seeds with ultrasound causes changes in the morphological characteristics of the sprouts (Table 3). The length of the embryo root compared to the control increased for Oscar and Plum tart varieties up to 6 minutes of treatment and reached 2.59 cm and 2.65 cm,

respectively, and for Purple flora it was 2.25 cm at 4 minutes. A significant reduction was found at 8 minutes of exposure, most pronounced in the variety Plum tart, with 46.22% lower than the control. These data are statistically significant. A similar trend is observed for hypocotyls length. For cultivars Purple flora and Oscar, treatment with 6 minutes increased the most length, by 19.94% and 14.89% over the control, respectively. In the other variety Plum tart, this increase is 39.80% and is at 4 minutes. Inhibition from treatment with 8 minutes was also found for this trait, with the reduction ranging from 13.85% (Plum tart) to 21.29% (Purple flora). Seedling fresh weight is a major factor in determining seed vigor (Copeland & McDonald, 2001). In the three studied varieties, it increases uniformly up to 6 minutes. The increase compared to the control was the highest for Plum tart at 41.09% and the weight reached 41.2 mg. It was lower compared to untreated seeds for all varieties in the 8-minute treatment. A strong positive correlation was found between seedling fresh weight and embryo root and hypocotyls length, except for hypocotyls length in Plum tart, which was also positive but middle.

Table 3. Morphological characteristics of gladiolus seedlings after application of ultrasound

№	Variants	Length of embryo root (cm)			Length of hypocotyls (cm)			Fresh weight of one seedling (mg)		
		Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart	Purple flora	Oscar	Plum tart
1	Control	1.87	1.43	1.55	3.76	2.47	3.04	37.1	30.0	29.2
2	2 min	2.19	1.47	2.28	3.90	3.42	3.25	37.2	31.1	33.6
3	4 min	2.25	2.14	2.13	4.11	3.67	4.25	38.6	32.8	33.6
4	6 min	2.04	2.59	2.65	4.51	4.32	3.44	41.4	35.3	41.2
5	8 min	1.34	0.96	1.06	3.10	2.16	2.67	33.5	28.2	28.4
LSD p = 0.05		0.4	0.6	0.71	0.41	1.1	0.39	1.1	2.4	2.8
r*		0.73	0.98	0.91	0.99	0.96	0.46			

r* Correlation coefficient with fresh weight of one seedling

CONCLUSIONS

The application of ultrasound to gladiolus seeds there is a pronounced effect on their sowing qualities, with a well-defined variety response observed. Germination energy and germination in most cases are increased the most from exposure to 6 minutes. Polynomial regression with high coefficients of determination was found between treatment duration and germination.

A positive influence of ultrasound treatment is reported on the overall vital status of gladiolus seeds, improving parameters such as mean germination time, uniformity of germination and the time for germination of 50% of the seeds. The development of seedlings is the best in treatment with 6 minutes. High inhibition causes ultrasound with a duration of 8 minutes.

It is recommended to apply a treatment of gladiolus seeds with 6 minutes of ultrasound. The obtained results can be predominantly used to improve seed development, especially in hybridization breeding programs. This will highly improve the research activities in hybrid selection, which will increase their effectivity.

REFERENCES

Aladjadjiyan, A. (2007) - Research the connections between vitality and physical properties at carrots (*Daucus carota* L.), Nantes variety, treated with ultrasound and laser rays. *Scientific works of Bulgarian Union of the Scienties*, III, 128-133.

Aladjadjiyan, A. (2007a). The use of physical methods for plant growing stimulation in Bulgaria. J*ournal Central European Agriculture*, 8(3), 369-380.

Azim, M. H. (2019). Progeny test of crosses among different cultivars of gladiolus. *Journal of plant production, agronomy, breeding and horticulture*, 41(4), 29-44

Barnard, T.T. (1972). On hybrids and hybridization. In: Lewis, G.J., Obermeyer, A.A. & Barnard, T.T.. Gladiolus, a revision of the South African species. *Journal of South Africa Botany*, 10, 304–310.

Basra, S.M.A., Pannu, S. & Afzal, I. (2003). Evaluation of seedling vigor of hydro and matriprimed wheat (*Tritium aestivum* L.) seed. *International Journal of Agricultural Biology*, 5, 121-123.

Black, M., Bewley, J. D. & Halmer, P. (2006). *The encyclopedia of seed science, technology and uses*. Cromwell Press, Trowbridge, UK, 828.

Bose, T.K., Yadav, L.P. & Pal, P. (2003). Gladiolus. In: *Commercial Flowers*. Department of Horticulture, Bidhan Chandra Krishi. Naya Prokash, 1-33.

Carpenter, W.J., Wilfret, G.J., Cornell, J.A. (1991). Temperature and relative humidity govern germination and storage of gladiolus seed. *Hortscience*, 26(8), 1054-1057.

Copeland, L. & Mcdonald, M. (2001). *Principles of seed science and technology*. Chapman & Hall, New York.

Farhat, T. (2004). *Plant characteristic and vase life of gladiolus flowers as influenced by the preharvest and npk application and postharvest chemical treatment*. M.Sc. (hons). thesis, Pmas-Aaur, 1-22.

Farooq, M., S., Basra, M. A., Nazir, A., Hafeez, K. (2005). Thermal hardening: a new seed vigor enhancement tool in rice. *Journal of Integrative Plant Biology*, 47(2), 187-193.

Fowel J., L. Cohen. (1992). *Practical statistics for field biology*. John Wiley & Sons, New York. 227 pages.

Goldblatt, P. & Manning, J. (1998). *Gladiolus in Southern Africa*. Fernwood press, Vleaberg, S. Africa, 212.

Horţ, D., Cantor, M., Buta, E. & Andriescu, I. (2012). Researches regarding intraspecific hybridization of *Gladiolus* L. Species in order to obtain novel ornamental varieties. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, 69(1), 172-177.

Huxley, A., Griffiths, M. & Levy, M. (1992). Gladiolus. In: eds. Griffiths, M. & Huxley, A.J *The new royal horticultural society dictionary of gardening*, New York: Stockton Press, 2, 25-45.

Kader M.A. (2005). A Comparison of Seed Germination Calculation Formulae and the Associated Interpretation of Resulting Data. *Journal & Proceedings of the Royal Society of New South Wales,* 138, 65–75.

Manning, J & Peter, G. (2008). The Iris Family. *Natural History & Classification*. Portland, Oregon, USA. Timber Press, 138–142.

Memon, S. A., Baloch, A. R., Baloch, M. A., Keerio, M. I. (2013). Pre-soaking treatment and foliar application of KNO_3 on growth and flower production of gladiolus (*Gladiolus hortulanus*). *Journal of Agricultural Technology*, Vol. 9(5): 1347-1366

Miano, A.C,. V.A. Forti, H.F. Abud, F.G. Gomes-Junior , S.M. Cicero and P.E.D. Augusto (2015). Effect of ultrasound technology on barley seed germination and vigour. *Seed Science and Technology*, 43(2): 1-6.

Mushtaq, S., Ishfaq, A. H., Syed ,Z., Ul, H., Muhammad, A., Muhammad, A. S., Rizwan R., Misbah, R., Muhammad, A., Muhammad, S. I. (2012). Evaluation of seed priming on germination of *Gladiolus alatus*. *African journal of biotechnology*, 11(52), 11520-11523,

Panayotov N. (2015). *Seed science and factors of seed production of the vegetable crops.* Academic press of Agricultural University-Plovdiv. 281.

Panchev, V. (2022). Influence of the ultrasound treatment on the sowing parameters of *Colutea arborescence. Proceeding of Agribalkan Congress*, Edirne, Turkey, 1041-1048

Patero, T. & Augusto, P.E.D. (2015). Ultrasound (US) enhances the hydration of sorghum (*Sorghum bicolor*) grains. *Ultrasonics Sonochemistry*, 23: 11-15.

Raj, S. K., Srivastava, A., Chandra, G. & Singh, B. P. (2009). Characterization of cucumber mosaic virus isolate infecting Gladiolus cultivars and comparative evaluation of serological and molecular methods for sensitive diagnosis. *Current science*, 83(9), 1132-1137.

Ramzan, A., Hafiz, I. A., Ahmad, T. & N. Abbasi, A. (2010). Effect of priming with potassium nitrate and dehusking on seed germination of gladiolus (*Gladiolus alatus*) *Pakistan Journal of Botany*, 42(1): 247-258.

Takatsu, Y., Kasumi, M., Manabe, T., Hayashi, M., Marubashi, W. & Niwa, M. (2001). Temperature Effects on Interspecific Hybridization between *Gladiolus* ×*grandiflora* and *G. tristis*. *Hortscience*, 36(2): 341-343.

Takatsu. Y., Suzuki, K., Yamada, T., Inoue, E., Gonai, T., Nogi, M., Kasumi, M. (2005). Interspecific hybridization of wild gladiolus species using a dendrogram based rapid analysis. *Proceeding of IX International Symposium on Flower Bulbs, ISHS*. Eds.: Okubo, H., Miller, W.B. & Chastagner, G.A., *Acta Horticulturae*, 673, 475-480.

Traynov, Al. (2021). *Productivity and quality of carrot seeds through optimization of the nutrient regime in their seed production. PhD thesis.* Agricultural University-Plovdiv, 218.