Analysis of germination of seeds of Capsicum annum, L. and Lycorersicum esculent, L. treated with growth-promoting compounds using fluorescence spectroscopy

Vanya Slavova[1]*, Veselina Masheva[2], Katya Uzundzhalieva[2], Nurettin Tahsin[3]
Agricultural Academy, [1]Maritsa Vegetable Crops Research Institute, Plovdiv, Bulgaria
Agricultural Academy, [2]Institute of Plant Genetic Resources "K.Malkov", Sadovo, Bulgaria
[3]Agrarian University, Plovdiv, Bulgaria
*E-mail: *vania_plachkova@abv.bg*

Citation
Slavova, V., Masheva, V., Uzundzhalieva, K., & Tahsin, N. (2022). Analysis of germination of seeds of Capsicum annum, L. and Lycorersicum esculent, L. treated with growth-promoting compounds using fluorescence spectroscopy. *Bulgarian Journal of Crop Science, 59(6)* 34-40.

Abstract
Seed germination is a complex process caused by the interaction of hormonal, metabolic, genetic, and environmental factors. The variability of this trait has a great influence on the efficient production of seedlings and the quality of the crop. The degree of seed germination can be influenced by various pre-treatments and the non-destructive measurement to determine it by fluorescence spectroscopy, which will allow the application of the method as non-invasive and fast-acting. To confirm the flexibility of the method, seeds of *Capsicum annum, L* and *Lycorersicum esculentum,* L were primed with sterile distilled water (SDW), giberilic acid (GA_3) and hydrogen peroxide (H_2O_2). A portable spectrometer, model AvaSpec-ULS2048CL-EVO, was used for the monitoring.

The results are confirmed by faster logical variations of seeds, treatment with H_2O_2 and GA_3 medium compared to seeds, and treatment with SDW for 3 consecutive days. The method can be used in various agricultural fields and shows its potential as a rigorous sensory method for rapid selection of optimal compounds that stimulate plant growth compared to standard methods.

Key words: fluorescence spectroscopy; seeds; germination

INTRODUCTION

Improving the sustainability of agricultural production and reducing its impact on the environment in order to meet the nutritional needs of a growing population is one of the greatest challenges facing humanity today (Edmondson et al., 2014).

Achieving the idea of agricultural sustainability is based on the need to develop technologies and practises along the entire production chain without adversely affecting environmental conditions while leading to improvements in food productivity. (Paparella et al., 2013).

The quality of seeds and planting material is one of the main highlights, which has a strong effect and can increase the yield significantly. On the one hand, quality seeds and their products are directly or indirectly related to human health. Seed germination is a complex process that is influenced by both internal (the seed's physiological and hormonal state) and external (environmental conditions during seed development, germination, and early seedling growth) factors, as well as harvesting and storage time. (Basnet et al., 2015; Finch-Savage & Bassel, 2016)

Successful early seedling production requires efficient germination, rapid, uniform emergence and growth. Conventional seed germination generally involves three distinct phases (Lutts et al., 2016), consisting of (1) Phase I: the seed hydration process associated with passive uptake of water from dry tissues and associated with water move-

ment that appears first in the apoplastic spaces; (2) Phase II: the activation phase associated with the re-establishment of metabolic activities and restorative processes at the cellular level; and (3) Phase III: the initiation of growth processes associated with cell elongation and leading to root protrusion.

Different methods and techniques have been developed and used that accelerate the phases and improve the processes, such as pre-sowing treatment-seed priming, leading to a physiological state that increases the speed, percentage of germination, and uniformity of seed germination. (Hussain et al., 2006)

Hydropriming is the simplest method of seed priming, which relies on soaking the seeds in clean water and re-drying them to the original moisture content before sowing. This method is cheap and environmentally friendly, as it does not require the use of additional chemical substances as a priming agent. The main disadvantage of hydropriming is the uncontrolled absorption of water by the seeds.

Osmopriming involves soaking the seeds in an osmotic solution. Due to the low water potential of osmotic solutions, water enters the seeds slowly, which allows gradual imbibition and activation of the early phases of germination but prevents the emergence of roots (Di Girolamo & Barbanti, 2012).

Chemical priming refers to the treatment of seeds with various chemical solutions used as priming agents. This approach includes priming with a wide range of natural and synthetic compounds such as antioxidants (ascorbic acid, glutathione, tocopherol, melatonin, and proline), hydrogen peroxide, sodium nitroprusside, urea, thiourea, mannose, selenium, chitosan, fungicide, etc. (Khaliq et al., 2015; Jisha & Puthur, 2016; Srivastava et al., 2010). Biopriming involves imbibition along with bacterial inoculation of seeds (Reddy, 2012).

Different research methods and techniques are applied to study these processes, but as a result, microscopic analysis is the only way to observe the germination process by measuring the length and fresh weight of seedlings and seeds, and the evaluation of quality indicators is a process that takes time. Therefore, the development of fast and accurate methods such as optical diagnostics based on non-destructive analysis will help to overcome the aforementioned barriers in studying and monitoring the processes related to seed germination and guarantee quality planting material and seeds for agro-industry and farmers (Huang et al., 2015)

The optoelectronic methods for assessing the quality of plant seeds are non-contact, fast-acting, selective, and do not destroy the integrity of the examined sample. On the basis of these, it is possible to create non-invasive methods for the evaluation of tomato and pepper seeds. Until now, there has been no data on their research using the proposed method. Belyakov (2019) obtained results in the study of cereal seeds. Based on his research, emission excitation wavelengths of 362 nm, 424 nm and 485 nm were established. In these studies, it was found that during the ripening of seeds of cereal plants (for example, wheat, oats, and corn), the ratio of their excitation levels and changes in radiation for immature seeds is characteristic of the short-wave range, and long-wave prevails in mature seeds. The dependence of the ratio of long-and short-wavelength fluxes on the maturation time increases and can be statistically reliably approximated by the linear functions required to create a database.

Belyakov et al. (2021) developed a sensor for determining the level of physiological maturity of seeds, allowing by irradiating seeds with two sources at certain wavelengths and recording the photoluminescent flow with appropriate receivers to determine the stage of seed maturation . The maximum luminescence is less pronounced than in the excitation spectrum.

The spectral luminescence characteristics of forage plant seeds were measured by scarification during the study. The spectral characteristics of the seeds increase, due to the scarification of forage plants. It was established that in the studied seeds with repeated scarification, the observed qualitative changes in the excitation spectrum were related to the appearance of a new maximum at a wavelength of 423 nm. Likewise, for tomato and pepper seeds treated with different compounds, the obtained results can be used to create a schematic fiber-optic configuration for characterization of planting material from them.

The excitation and photoluminescence spectra of seeds of agricultural plants, legumes (Su et all, 2019) and tomatoes (Li et al., 2015) were measured using a previously developed method. The typical excitation spectrum was found to be in the range of 355–500 nm and to have two maxima: the main one at 424 nm and the side one at 485 nm. The emission

spectrum is in the range of 420–650 nm and has a maximum in the region of 500-520 nm.

The purpose of the study is to introduce fluorescence spectroscopy as a sensory method for research depending on the pre-sowing treatment (priming) of the seeds and the possibilities of its use as a potentially useful tool in the field of agriculture.

MATERIAL AND METHODS

Pepper seeds *Capsicum annum* L. and tomato seeds *Lycorersicumes culentum L*. obtained at the Institute of Plant Genetic Resources "K. Malkov", Sadovo, were included in the study.

The experiment was conducted in laboratory conditions (27.4°C; relative humidity of 80%), and the results were obtained within 14 consecutive days. The experiment was performed with 60 seed samples in three replicates (3 × 20) divided into three groups. All selected seeds are of equal initial fresh weight and placed for one hour in the respective solution.

The first group of 20 seeds was treated with gibberellic acid GA_3 (500ppm). The second group was treated with hydrogen peroxide H_2O_2 (3%), the remaining 20 seeds with sterile distilled water (SDW). The germination of the different groups of seeds was determined for each day of the study period.

Seed germination (%) - ratio of the total number of normally germinated seeds to the total number of seeds.

Fluorescence spectroscopy was used to determine the effect of hormonal and chemical priming of tomato and pepper seeds.

The research was carried out with a fiber-optic spectrometer, which allows the generation of emission fluorescence signals from 200 nm to 1200 nm. The device is used for fluorescence spectroscopy of solid samples with a photosensitive area of 1.9968×1.9968 mm.

The experimental setup includes a laser diode (emission wavelength 285 nm, optical power 16 mW, DC), a portable spectrometer model AvaSpec-ULS2048CL-EVO. The sample (petri dish with treated tomato and pepper seeds) is placed on a duralumin stand, which allows reception of an emission signal from it under 180° by a U-shaped optical fiber. This reduces aberrations and allows the generation of a better quality emission fluorescence signal (Fig.1). The resolution of the spectrometer can be in the range of 0.06 - 20 nm, with that of the setup used for our experiment being 0.09 nm. It is preferable to use a laser diode (LED) as a source in the circuit, since its spectral

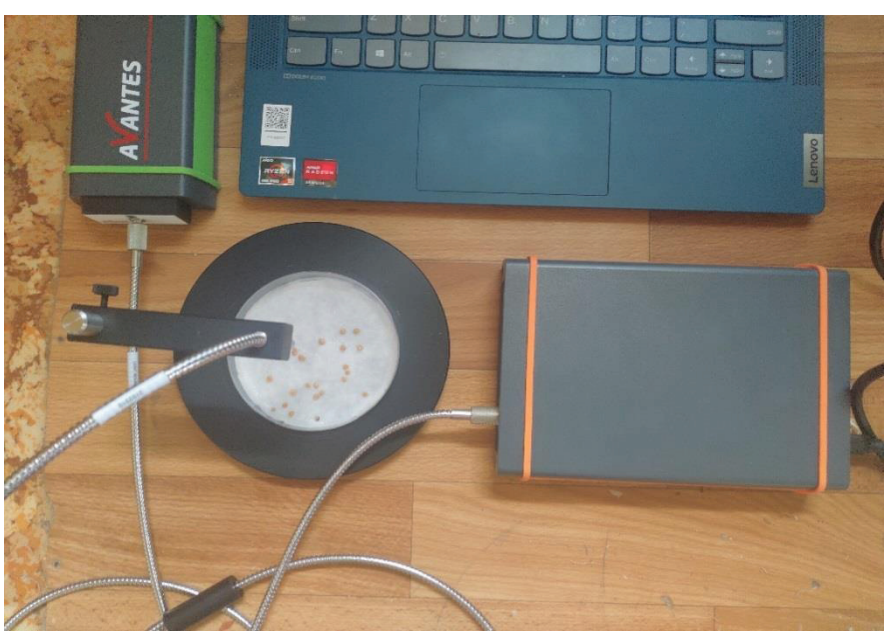

Figure 1. General view of the experimental installation used by fluorescence spectroscopy

width is very small. The LED used in the experiment has a relatively wide emission spectral width of about 30-40 nm and the angular distribution of its emission is in a large angular range of +/-300. The sensitivity of the spectrometer is in the range of 200 nm to 1200 nm. Its resolution is $\delta\lambda$= 5 nm.

The spectral setup based on fluorescence signals allows recording both the emission spectrum and the spectrum of the excitation source. The emission spectrum represents the wavelength distribution of an emission measured at a constant excitation wavelength. The excitation spectrum is the dependence of the emission intensity measured for one scanning wavelength against the excitation wavelength. This spectrum is represented as a function of the wavelength of the light intensity incident on the photodetector in the spectrometer.

For the particular circuit, the photodetector is of the CMOS model S9132 type. It was chosen because it can detect emission radiation from a sample with a very low content of a particular chemical compound.

The laser radiation is deflected from the source and hits the specimen. After the sample fluoresces, the emission signal is incident on a U-shaped optical fibre with a core diameter of 200 µm a step index of refraction, and a numerical aperture of 0.22. It takes him to the detector. In the spectrometer, the light signal is converted to electrical-digital using a USB 2.0 wire, downloaded to a computer with AvaSoft8 software, and exported to Excel. This allows analysis, processing, and visualisation of the results of the conducted research.

RESULTS AND DISCUSSION

The results for seed germination are presented in Figure 2. From the figure, it can be seen that GA_3 H_2O_2 treated (primed) seeds started the germination process earlier. In tomatoes, on day 4, 25% germination was reported for H_2O_2-treated seeds and 20% for GA_3-primed seeds. A similar trend was observed on the 6th day of the experiment in pepper seeds. The difference in reaching maximum germination between the treated tomato and pepper seeds and the control was 30% and 20%, respectively.

These results are in agreement with those obtained by Li et al. (2018), where it was stated that H_2O_2 and GA are essential for tobacco seed germination, and lowering the ABA/GA ratio induced mobilization and promoted seed germination. Studies in many species have shown that ABA and GA are major regulatory factors in seed dormancy and germination by triggering GA biosynthesis and ABA catabolism, and the antagonistic crosstalk between them plays a major role in modulating germination (Liu et al., 2010; Maarouf et al., 2015).

In addition to the conventional methodology, the method of fluorescence spectroscopy was also used in the present study. From the literature search, we have not found any results for the described experimental approach for the analysis of tomato and pepper seeds treated with GA_3, H_20 and H_2O_2. This gives us reason to claim that, for the first time, fluorescence spectroscopy has been applied as a sensing method for the rapid selection of optimal plant growth-stimulating compounds.

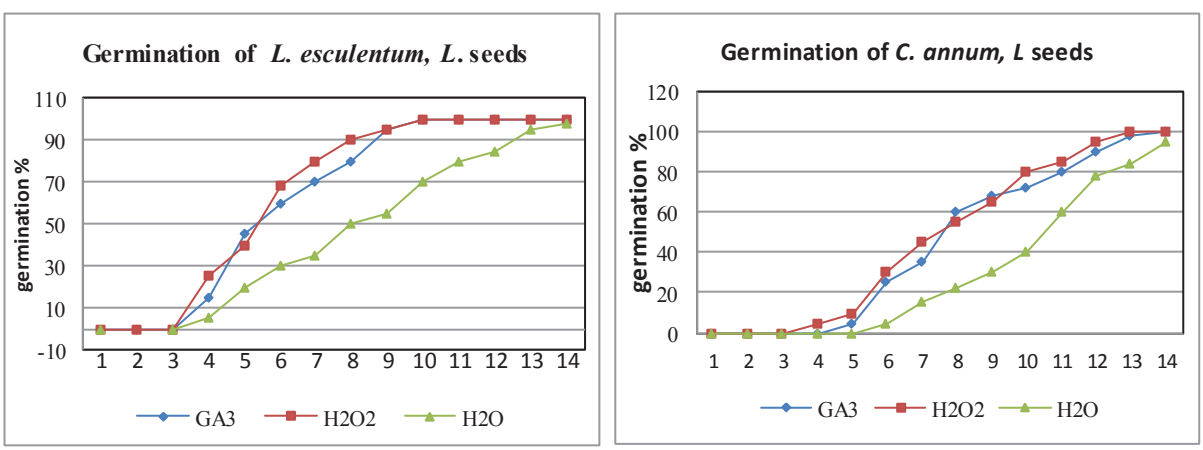

Figure 2. Germination (%) of *Lycorersicumes culentum* L., and *Capsicum annum L.* seeds

In Fig. 3 the emission wavelengths of tomato and pepper seeds treated with GA_3, H_2O_2 and SDW are presented. A clear difference was observed in the spectral distribution of the emission fluorescence signal between them.

As shown in Fig. 2 (a-tomatoes), the strongest peak is observed at 413 nm. The spectral profiles of the control and treated seeds were identical in the range of 350–510 nm. The large differences between the profiles are observed from 400 to 450 nm. Figure 2 (b-pepper) shows the spectral profiles of pepper seeds. The maximum of fluorescence emission were observed at 450 nm. Both types of seeds are most affected by H_20_2, with those from pepper being more pronounced. The spectral distributions of the treated tomato seeds showed higher intensity in contrast to those of pepper.

Fig. 4 shows tomato and pepper seeds treated with GA_3. A clear difference was observed in the spectral distribution of the emission fluorescence signal between them. The spectral distributions of the treated tomato seeds show a higher intensity in contrast to those of the pepper.

Fig. 5 shows tomato and pepper seeds saturated with H_20_2. A very small difference was observed in the spectral distribution of the emission fluorescence signal between them. The spectral distributions of the treated tomato and pepper seeds show similar signal intensities, which in turn leads to the conclusion that H_20_2 treatment leads to stimulation of the germination and growth process, as in pepper seeds *Capsicum annum L.*, as well as with tomatoes *L.esculentum L.*

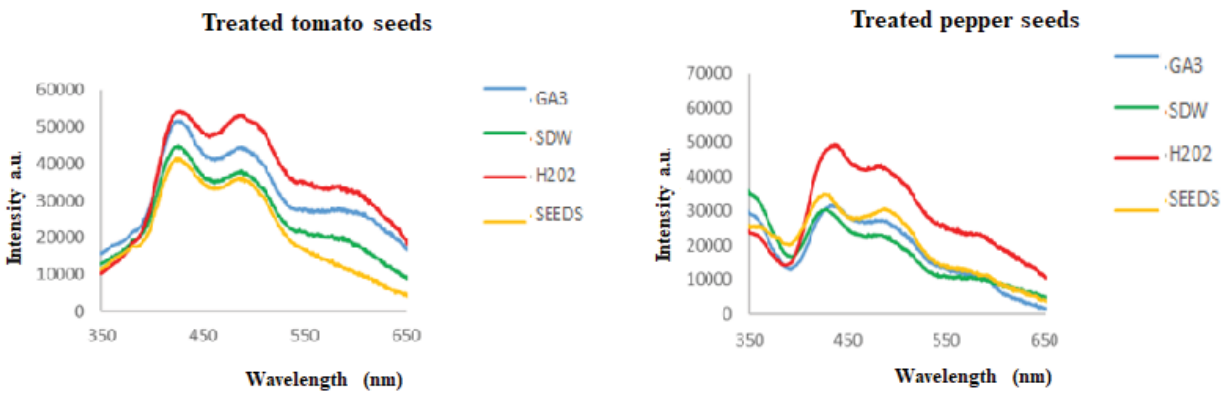

Figure 3. Emission wavelengths of tomato and pepper seeds treated with GA_3, H_20_2 and SDW

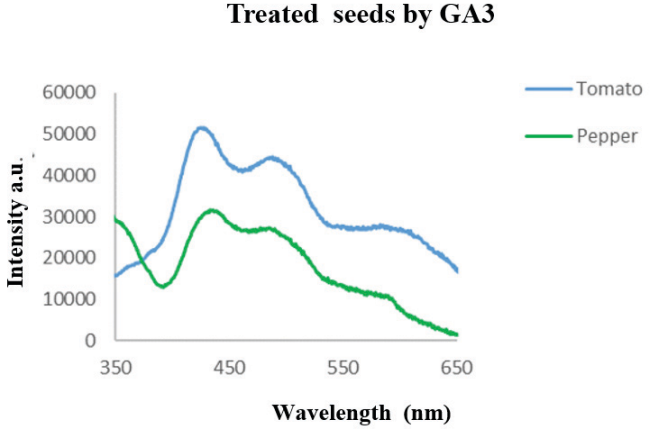

Figure 4. Emission wavelengths of tomato and pepper seeds treated with GA_3

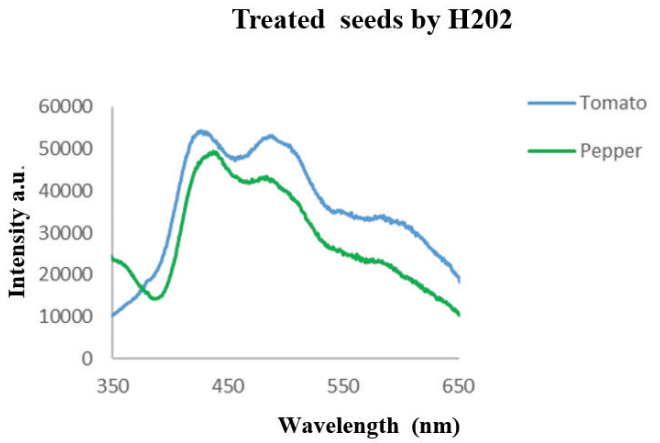

Figure 5. Emission wavelengths of tomato and pepper seeds treated with H_2O_2

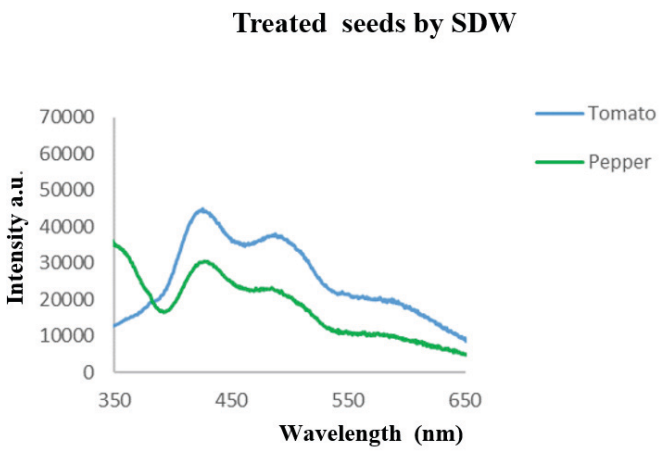

Figure 6. Emission wavelengths of tomato and pepper seeds treated with SDW

Fig. 6 presents the results for the untreated control tomato seeds (sterile distilled water, SDW). A difference was found between the spectral distribution of the emission fluorescence signal of the two types of seeds. Regardless of the higher intensity of the spectral distribution in the tomato seeds, they are significantly lower in intensity than the GA_3 and H_2O_2 treated seeds, and the same is true for the treated pepper seeds.

CONCLUSION

The systems engineering approach of adjustment (optical setting up) a specialized fluorescence spectroscopy applied research setup was found to be applicable in the analysis of tomato and pepper seeds treated with a specific stimulating compound.

A non-destructive method for evaluating pretreated tomato and pepper seeds is introduced and its advantages over conventional tests are demonstrated

With a sufficiently well-structured data library, fluorescence spectroscopy can be applied to analyze seeds treated with unknown compounds and infer the content of a particular compound or compilation of compounds.

REFERENCES

Basnet, R. K., Duwal, A., Tiwari, D. N., Xiao, D., Monakhos, S., Bucher, J., ... & Maliepaard, C. (2015). Quantita-

tive trait locus analysis of seed germination and seedling vigor in Brassica rapa reveals QTL hotspots and epistatic interactions. *Frontiers in plant science*, *6*, 1032.

Belyakov, V. M. (2019). Photo luminescence monitoring of ripeness of seeds of grain during the ripening process. *Inzhenernyetekhnologii i sistemy* 2, pp. 306-319.

Belyakov, M., Sokolova, E., Listratenkova, V., Ruzanova, N., & Kashko, L. (2021). Photoluminescent control ripeness of the seeds of plants. In *E3S Web of Conferences* (Vol. 273, p. 01003). EDP Sciences.

Di Girolamo, G., & Barbanti, L. (2012). Treatment conditions and biochemical processes influencing seed priming effectiveness. *Italian Journal of Agronomy*, *7*(2), e25-e25.

Finch-Savage, W. E., & Bassel, G. W. (2016). Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of experimental botany*, *67*(3), 567-591.

Edmondson, J. L., Davies, Z. G., Gaston, K. J., & Leake, J. R. (2014). Urban cultivation in allotments maintains soil qualities adversely affected by conventional agriculture. *Journal of Applied Ecology*, *51*(4), 880-889.

Huang, M., Wang, Q. G., Zhu, Q. B., Qin, J. W., & Huang, G. (2015). Review of seed quality and safety tests using optical sensing technologies. *Seed Science and Technology*, *43*(3), 337-366.

Hussain, M., Farooq, M., Basra, S. M., & Ahmad, N. (2006). Influence of seed priming techniques on the seedling establishment, yield and quality of hybrid sunflower. *International Journal of Agriculture and Biology*, *8*(1), 14-18.

Jisha, K. C., & Puthur, J. T. (2016). Seed priming with BABA (β-amino butyric acid): a cost-effective method of abiotic stress tolerance in *Vigna radiata* (L.) *Wilczek*. *Protoplasma*, *253*(2), 277-289.

Khaliq, A., Aslam, F., Matloob, A., Hussain, S., Geng, M., & Wahid, A. (2015). Seed priming with selenium: consequences for emergence, seedling growth, and biochemical attributes of rice. *Biological trace element research*, *166*(2), 236-244.

Li, B., Wang, H., Zhao, Q., Ouyang, J., & Wu, Y. (2015). Rapid detection of authenticity and adulteration of walnut oil by FTIR and fluorescence spectroscopy: A comparative study. *Food Chemistry*, *181*, 25-30.

Li, Z., Gao, Y., Zhang, Y., Lin, C., Gong, D., Guan, Y., & Hu, J. (2018). Reactive oxygen species and gibberellin acid mutual induction to regulate tobacco seed germination. *Frontiers in plant science*, *9*, 1279.

Liu, Y., Ye, N., Liu, R., Chen, M., & Zhang, J. (2010). H2O2 mediates the regulation of ABA catabolism and GA biosynthesis in Arabidopsis seed dormancy and germination. *Journal of experimental botany*, *61*(11), 2979-2990.

Lutts, S., Benincasa, P., Wojtyla, L., Kubala, S., Pace, R., Lechowska, K., ... & Garnczarska, M. (2016). Seed priming: new comprehensive approaches for an old empirical technique. *New challenges in seed biology-basic and translational research driving seed technology*, 1-46.

Maarouf, B., Sajjad, Y., Bazin, J., Langlade, N., Cristescu, S. M., & Balzergue, S. (2015). Reactive oxygen species, abscisic acid and ethylene interact to regulate sunflower seed germination. *Plant Cell Environment, 38*, pp. 364-374.

Paparella, S., Araújo, S. S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: state of the art and new perspectives. *Plant Cell Rep.* 34, 1281–1293. Doi: 10.1007/s00299-015-1784-y.

Reddy, P. P. (2012). Bio-priming of seeds. In: *Recent advances in crop protection* (pp. 83-90). Springer, New Delhi.

Srivastava, A. K., Lokhande, V. H., Patade, V. Y., Suprasanna, P., Sjahril, R., & D'Souza, S. F. (2010). Comparative evaluation of hydro-, chemo-, and hormonal-priming methods for imparting salt and PEG stress tolerance in Indian mustard (Brassica juncea L.). *Acta Physiologiae Plantarum*, *32*(6), 1135-1144.

Su, W. H., Fennimore, S. A., & Slaughter, D. C. (2019). Fluorescence imaging for rapid monitoring of translocation behaviour of systemic markers in snap beans for automated crop/weed discrimination. *Biosystems engineering*, *186*, 156-167.