INFLUENCE OF OZONE ON *IN VITRO* PLANTS

Līga Lepse

Pūre Horticultural Research Centre, Abavas iela 2, Pūre, Tukuma nov., LV -3124, Latvia. liga.lepse@puresdis.lv

Introduction

Ozone is presenting in low concentrations in Earth atmosphere and more often is mentioned as air pollutant, but at the same time it is broadly used in different industrial applications due to its high oxidizing properties. Well known property of ozone is ability in certain concentrations to destroy alive organisms. Destructive nature of ozone is caused by its ability to snap double bonds of organic molecules (Purich and Allison, 2000). In plants ozone reduces total chlorophylls, carotenoids and carbohydrate concentration, and increases amounts of functionally active compounds and production of ethylene. These properties of ozone are used in the destroying of organic pollution where severing of carbon-carbon bonds is useful for eradication of micro organisms (yeast, fungi, bacteria) – in postharvest technologies, food industry, disinfection of laboratories and medical equipment. There was not found any literature about ozone influence on virus eradication.

One of the main tasks of plant microclonal propagation *in vitro* is to eliminate viruses and other pathogen organisms from plants in order to obtain healthy and high quality planting material. For elimination of some pathogens it is enough just by cutting small size explants (usually 0.5 – 1 mm) and culturing them on appropriate media (Cassels, 2011). But some viruses, such as *Raspberry Bushy Dwarf Virus* (RBDV) for raspberries (*Rubus idaeus* L.) are complicate to eliminate from plants by routine *in vitro* methods. Several chemical components are used with different success to eliminate viruses (Pūpola et al., 2009). Ozone could be used as virus eliminating agent due to its destructive reaction on organic compounds, but in the same time in specific concentrations it has destructive influence also on plants. Therefore the preliminary investigation was carried out with the aim to observe surviving rate of *in vitro* plants after ozone treatment.

Methods

Investigation was carried out in Pūre Horticultural Research Centre, Latvia. *In vitro* plants of raspberry cultivar `Babye Leto – 2` were used in the trial. Microplants were grown in 200 ml glass jars, 6 microplants per jar. Ozone was obtained by ozone generator "BNP" SOZ-YOB-10g. Plants were treated by ozone in two ways: 1) 3 mg ozone gas was blown into 200 ml *in vitro* containers directly from generator and 2) microplants were immersed in ozonated water (O_3 concentration 0.25 ppm) for 1 hour and then transplanted into fresh medium in glass tubes of 25 ml volume, one plant per tube.

Plants were evaluated visually and the percentage of alive plants was calculated after 10 days.

Results and Discussion

Plants reacted very differently in both treatments. Plants immersed in the ozonated water did not show any signs of stress or some disorders at the moment of treatment and also after 10 days. 95 % of microplants continued their growing into tubes after ozone treatment and transplanting without significant losses. There were 5% of all plants showing fungal infections.

Plants ozonated with O_3 gas reacted differently. At the moment of ozone treatment leaves loosed chlorophyll and became whitish, without any turgor signs. However after 10 days microplants developed 3-4 leaves from the apex, which obviously was remain alive and able to develop leaves from primordia. The percentage of transplanted microplants in next passage was 102 % (some plants were clustered and by division were multiplied). So we can conclude that used ozone concentration was enough critical to destroy chlorophyll and collapse the most gentle (thin and soft) parts of plant exposed to direct influence of O_3, but did not destroy apical meristem and leaf primordia. This allows us to assume that there is a high probability that also pathogen organisms could be destroyed. The evaluation of ozone effectiveness on elimination of plant pathogens will be investigated in the next phase of investigations.

Obtained results are close to those referred from other investigations and observations about ozone influence on alive organisms. Reduced photosynthesis and plant biomass after ozone treatment for strawberry plants exposed to 85 ppb ozone (8 h per day) for 40 days is referred by Drogoudi and Ashmore (2002). Destroying of plant pigments after ozone treatment is referred by Iglesias et al. (2006) and Song et al. (2003). They refer about different responses of plants according to concentration and duration of ozone application – the highest doses causes carrot peel discoloration during storage and reduced total chlorophylls, carotenoid and carbohydrate concentration for Clementina mandarin plants. In the same time appropriate concentrations, temperature and duration of ozone application reduces contamination with microorganisms for different fresh commodities (Kim et al., 2006; Das et al., 2010).

Conclusions

Ozonated water at 0.25 ppm concentration has no any visible influence on raspberry microplants after their soaking in the water.

Ozone gas application directly in the growing jar of raspberry microplants (3 mg O_3 per 200 g jar) has instant influence on microplants – leaves become whitish and loose their turgor, but apex of the plant stays alive and keeps plant ability to develop new leaves and continue vegetation.

Both tested ozone application methods can be used in future investigations of virus elimination from microplants *in vitro*.

References

1. Cassells, A.C., 2011. Detection and elimination of microbial enfophytes and prevention of contamination in plant tissue culture. p. 223-238. In: R.N. Trigiano and D.J. (eds.), Plant tissue culture, development, and biotechnology. GrayCRC Press, New-York.
2. Das, B.K., Ji Gang Kim and Ji Weon Choi, 2010. Role of different washing solutions and contact time on the microbial quality and food safety of fresh-cut paprika. Acta Hort., 875:283-290.
3. Drogoudi, P.D. and Ashmore, M.R., 2002. Screening of three strawberry cultivars for their ozone sensitivity. Acta Hort., 579: 275-280.
4. Iglesias D.J., Calatayud Á., Barreno E., Primo-Millo E., Talon M., 2006. Responses of citrus plants to ozone: leaf biochemistry, antioxidant mechanisms and lipid peroxidation. Plant Physiology and Biochemistry, 44 (1-2): 125-131.
5. Kim, B.S., Kwon, J.Y., Kwon, K.H., Cha, H.S., Jeong, J.W. and Kim, G.H. 2006. Antimicrobial effect of cold ozonated water washing on fresh cut lettuce. Acta Hort., 699:235-242
6. Pūpola N., Lepse L., Kāle A., Moročko-Bičevska I., 2009. Occurrence of RBDV in Latvia and virus elimination in vitro by chemotherapy. Horticulture and vegetable growing, 28(3): 165-172.
7. Purich D.L., Allison R.D., 2000. Handbook of biochemical kinetics. p. 532-533. Academic Press, New York.
8. Song J., Fan L., Forney C.F., Hildebrand P.D., Jordan M.A., Renderos W., McRae K.B., 2003. Ozone and 1-MCP treatments affect the quality and storage life of fresh carrots. Acta Hort. 628: 295-301.