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Response of *Phalaenopsis* Orchid to Selenium and Bio-Nano-Selenium: *In Vitro* Rooting and Acclimatization

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ORCHID plants are considered one of the most valuable and largest flowering plant families, which have a premium position in the global market of cut-flowers. Rooting of orchids is still the main real problem that obstructs its propagation, as roots are hardly to develop.So, Orchid needs to improve its *in vitro* propagation by enhancing its rooting development. The plant tissue culture of orchids is the most promising field that has been commercially used for getting of virus-free and high-quality rooted plants and representing around 70% of transplants production. In this study the shoots of *in vitro* plantlets were separated and cultured on half MS medium supplemented with nano-Se (0,10, 20, 30, 40 and 50 mg l^{-1}) or corresponding doses of bulk type selenite; sodium selenite (0, 0.5, 1, 2, 4, 6, 8 and 10 mg l^{-1}) in separates treatments with or without 0.5 mg l^{-1} NAA (α-naphthalene acetic acid). The two different sources of selenium were selected at mentioned concentrations to improve orchid rooting and acclimatization. Supplementation of half MS medium with 50 mg l^{-1} nano-Se + 0.5 mg l^{-1} NAA recorded the highest significant values of rooting percentage and root length as well. Whereas, half MS medium fortified with 40 mg l^{-1} nano-Se + 0.5 mg l^{-1} NAA gave the optimum number of roots. On the other hand, this enhancing effect on rooting decreased after augmentation the culture medium with 10 mg l^{-1} selenite. It could be concluded that *in vitro* rooting of *Phalaenopsis* orchid was improved by supplementation of nano-Se up to 50 mg l^{-1} in presence of 0.5 mg l^{-1} NAA consequence, acclimatization process was enhanced.

Keywords: Moth orchid, Cut-flowers, MS medium, Nano-selenium, Sodium selenite.

Introduction

Orchid plants belong to family Orchidaceae which represents one of the two largest plant families (6-11% of flowering plant species), which include 736 – 899 genera, 27,800 species and more than 100,000 hybrids worldwide (Cardoso et al. 2020). Orchids also have several benefits besides its unquestionable importance from the botanical and ecological aspects, the most important cut-flowers in the world commercial floriculture and for medicinal purposes (*e.g.,* some species of the genera *Dendrobium*, *Gastrodia* and *Bletilla*) (Cardoso et al. 2020). It is reported that orchids have a significant share of the annual sales of the global flower trade more than 4 billion US $ (Zhang et al. 2018). The moth orchid (*Phalaenopsis* spp.) is known as the most popular orchid, cut flowers and potted plants worldwide. It takes about 15–18 months to start flowering (Zhou et al. 2018 and Cho et al. 2020). The hybrids of *Phalaenopsis* represent one of the most widespread orchids in flower market due to their long-lived and spectacular flowers. *Phalaenopsis* also has special agronomic characters due to their abundant flower color, including yellow, red-purple and white. Concerning the red-purple

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flowers, they have a series in colors including purple, violet, red-purple, purpleviolet and violet-blue (Liang et al. 2020).

The seed germination of orchids and its biology are still extremely complex and varied between orchid species (Rasmussen et al. 2015). The seeds of orchid species do not have nutritional reserves and the endosperm is absent from the mature orchid seeds (Yeung et al. 2018). Both the seedlings and seed embryos are mainly depending on the symbiosis food relationship with the mycorrhizae, which have the ability to nutritionally supply these plant species at early germination and for a long time till the complete establishment of the seedling under the natural environments (Cardoso et al. 2020).The micro-propagation and traditional breeding have been used in several orchids to get the cut flowers and/or potted plants in horticultural markets (Wang et al. 2019). Due to the growth nature of orchid roots, their rooting difficulty is one of the main problems facing propagation and production of orchids (Zhang et al. 2018). Concerning production of *Phalaenopsis*, the high difficulty of *in vitro* rooting is considered one of the most barriers facing the micropropagation of this plant. This obstacle may increase the production cost of micro-propagated plantlets of *Phalaenopsis*, which might lead to fall its profits in the international market (Cardoso et al. 2020). It is worth mention that the germination rates of orchids *in vitro* are reported to be higher than 70%, whereas this rate in germinated seeds may hardly exceed 5% under the natural conditions and in *ex vitro* conditions (Cardoso et al. 2020).

Selenium (Se) can counteract many stressful conditions, which adversely damage cultivated plants including biotic and abiotic stress (Hasanuzzaman et al. 2020) such as high temperature (Hawrylak-Nowak et al. 2018), drought (Sattar et al. 2019), waterdeficit (Sattar et al. 2019), salinity (Kamran et al. 2020) and heavy metal stress (Feng et al. 2021; Huang et al. 2021). Under *ex vitro* conditions, nano-Se has been used in ameliorating different stresses such as high temperature (Djanaguiraman et al. 2018; Seliem et al. 2020), salinity (Morales-Espinoza et al. 2019 and Zahedi et al. 2019), heavy metals stress (Hussain et al. 2020) and biotic stress (Quiterio-Gutiérrez et al. 2019). Few studies have been published concerning the role of Se in promoting *in vitro* plant growth under stressful conditions but a very fewer regarding nano-Se under *in vitro* conditions (Domokos-Szabolcsy et al. 2012, 2014; El-Ramady et al. 2013, 2014 a,b; Zsiros et al. 2019).

It has been hypothesized that Se applications in mineral form (sodium selenite) or nano form (nano-Se) could be reliable for shooting and rooting of *Phalaenopsis* orchid *in vitro* depending on the Se source and its concentration. Therefore, this is the first report that reviewed the comparison between the biological role of inorganic selenium and bio nano-selenium on supporting the *in vitro* shooting, rooting and the acclimatization of *Phalaenopsis* under *ex vitro* conditions. We need to answer the following basic question regarding the stimulator impact of Se and nano-Se: can Se-forms enhance the rooting and acclimatization of *Phalaenopsis* orchid?

Materials and Methods

Plant materials

In vitro plantlets of *Phalaenopsis* spp. were cultured on half MS (Murashige and Skoog 1962) solidified medium containing 15 g l^{-1} sucrose, 8.0 g l^{-1} agar and supplemented with 10 mg l^{-1} BA (benzyl adenine) + 1 mg l^{-1} NAA. Cultures were kept at 25°C, 40 µmol m^{-2} s^{-1} photosynthetic photonflux density (PPFD), photoperiod (16 h/d) for 4 weeks for shoot multiplication. The produced shoots were separated individually then cultured on MS medium free growth regulators for shoot elongation. After 3 weeks of culture, shoots of about 3.0 cm lengths were used for the following experiments.

Selenium treatments

Shoots were cultured on half MS medium with 15 g l^{-1} sucrose and 8.0 g l^{-1} agar. A series of Se treatments were selected using two different sources of selenium at different concentrations based on the previous literatures as follows:biological nano-selenium (BioNano-Se) at 0, 10, 20, 30, 40 and 50 mg l^{-1} and sodium selenite at 0, 0.5, 1, 2, 4, 6, 8 and 10 mg l^{-1}. The biological nano-Se was prepared according to Eszenyi et al. (2011). The previous concentrations of both Se-sources were added separately to MS medium with/without 0.5 mg l^{-1} NAA. The pH of the medium was adjusted to 5.8 by 0.1 N KOH or HCl before autoclaving at 121 °C and 1.2 kg cm^{-2} pressure for 15 min.Three cm length shoots were cultured in 250 ml jar containing 30 ml medium. Cultures were maintained in light at 40 µmol m^{-2} s^{-1} PPFD. Shoot length, number of shoots, number of leaves, rooting percent, root length and number of roots were recorded after 6 weeks of culture. The source of biological nano-Se was kindly provided by the Agricultural Microbiology Department, Soils, SWERI, Kafr El-Sheikh, Egypt, whereas the source of sodium selenite (Na_2SeO_3, Sigma-Aldrich, Saint Louis, USA) was kindly provided by Nano-Food Lab, Debrecen University, Hungary.

Ex vitro acclimatization

Plantlets at the 3-4 leaf stage were transplanted into culture pots (5 cm) filled with sterilized peat moss. The plantlets were grown in air-conditioned greenhouse. The environment in the greenhouse was adjusted to a 25 ± 2°C air temperature, 40 – 50% relative humidity and a 100 µmol m^{-2} s^{-1} PPF with a 16 h photoperiod using halide lamps. Survival percentage was recorded after 10 weeks.

Statistical analyses

The authors clarify that the quality assurance for obtained results was achieved during the performance of this study by applying the internal and external quality assurance systems at the Physiology and Breeding of Horticultural Crops Laboratory (Accredited according to ISO/17025), Dept. of Horticulture, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh,Egypt. The experiments were set up in a completely randomized design in two replicates. The means and ANOVA were calculated using SPSS (version20) statistical software. All the obtained data of this study were tabulated and statistically analyzed using Duncan's Multiple Range Test for comparing among means of different treatments according to Snedecor and Cochran (1990) and significance was determined at p≤ 0.05.

Results

In vitro shooting of Phalaenopsis orchid

In the current study, *in vitro* shooting of *Phalaenopsis* was evaluated using half MS medium with/without NAA and augmented with different concentrations of nano-Se or Se. Shooting measurements included shoot length, number of shoots and number of leaves. The obtained results clearly showed that all shooting parameters were significantly influenced by supplementation of Se or nano-Se at different applied concentrations except number of shoots. For nano-Se, the maximum significant value of shoot length (7.3 cm) was recorded for 50 mg l^{-1} nano-Se + 0.5 mg l^{-1} NAA,whereas the highest number of leaves (8) was noticed for 50 mg l^{-1} nano-Se without NAA. On the other hand, there weren't significant differences among nano-Se treatments on number of shoots (Table 1).

About Se**,** the highest shoot length (6 cm) was achieved on 4 mg l^{-1} Se + 0.5 mg l^{-1} NAA followed by 1 mg l^{-1} Se without NAA, without significant differences between them. Also, the best result for number of leaves (7.7) was registered on 1 mg l^{-1} Se without NAA. As noticed for nano-Se, numbers of shoots did not record significant differences under Se treatments (Table 2).

TABLE 1. ***In vitro*** **shooting of** ***Phalaenopsis*** **orchid on half MS medium with/without NAA and supplemented with different concentrations of nano-selenium**

1/2 MS +		Shoot length (cm)	Number of shoots	Number of leaves
Nano-Se (mg l^{-1})	NAA (mg l^{-1})			
0	0	4.8 bc	1.3	4.3 bc
10	0	5.0 b	1.0	5.0 b
20	0	4.7 bc	1.3	4.0 bc
30	0	4.2 c	1.3	6.0 ab
40	0	5.7 b	1.0	5.7 b
50	0	5.0 b	1.0	8.0 a
0	0.5	6.2 ab	1.0	3.3 c
10	0.5	4.8 bc	1.7	4.6 bc
20	0.5	5.3 b	1.0	6.0 ab
30	0.5	5.8 b	2.0	6.0 ab
40	0.5	6.3 ab	1.0	5.0 b
50	0.5	7.3 a	1.0	5.6 ab
F-test		*	**NS**	*

Different letters in the same column show significant differences among treatments according to Duncan's test at p ≤ 0.05, NS: non-significant differences, NAA: α-napthalene acetic acid.

TABLE 2. *In vitro* shooting of *Phalaenopsis* orchid on half MS medium with/without NAA and supplemented with different concentrations of selenium

1/2 MS + Bulk Se (mg l^{-1})	NAA (mg l^{-1})	Shoot length (cm)	Number of shoots	Number of leaves
0	0	3.7 b	1	3.0 d
0.5	0	5.2 ab	1	4.0 cd
1	0	5.8 a	1	7.7 a
2	0	4.3 b	1	4.7 bc
4	0	3.5 b	1	4.0 b
6	0	5.0 ab	1	6.3 ab
8	0	4.3 b	1	4.3 c
10	0	3.8 b	1	6.0 b
0	0.5	5.2 ab	1	3.0 d
0.5	0.5	4.3 b	1	4.3 c
1	0.5	5.2 ab	1	4.3 c
2	0.5	4.5 b	1	5.3 c
4	0.5	6.0 a	1	5.0 b
6	0.5	4.7 b	1	3.3 d
8	0.5	4.5 b	1	4.3 c
10	0.5	3.5 b	1	5.3 c
F-test		**	NS	**

Different letters in same column show significant differences among treatments according to Duncan's test at $p \leq 0.05$, NS: non-significant differences, NAA: α-napthalene acetic acid.

In vitro rooting of Phalaenopsis orchid

The rooting of *Phalaenopsis* orchid *in vitro* under different applied concentrations of Se or nano-Se to half MS medium with and without NAA was investigated. In general, there were significant differences among nano-Se or Se treatments on rooting percentage and root length as well. In case of nano-Se, 50 mg l^{-1} nano-Se + 0.5 mg l^{-1} NAA recorded not only the optimum rooting percentage (100%) but also the highest root length (8.5 cm), without significant differences between it and some treatments. whereas the best result for number of roots was obtained on 40 mg l^{-1} nano-Se + 0.5 mg l^{-1} NAA (Table 3 and Fig. 1 & 3).

Concerning Se, the maximum rooting percentage and root length (100% and 6.3 or 6.2 cm, respectively) were obtained on 1 mg l^{-1} Se with or without NAA, respectively which appeared to be better than 2 mg l^{-1} Se without NAA, where there are no significant differences between them. As, lower Se concentration is preferable than the high one. While the highest number of roots was noticed on 4 mg l^{-1} Se + 0.5 mg l^{-1} NAA (Table 4 and Fig. 2 & 4).

Ex-vitro acclimatization of Phalaenopsis orchid

The results of acclimatization clearly indicated that all examined nano-Se treatments achieved survival percentages approached or reached to 100% except zero nano-Se treatment (Fig. 5). But in case of Se treatments, most Se treatments alone or combined with NAA recorded 100% survival percentage (Fig. 6) except zero, 10 mg l^{-1} Se and 10 mg l^{-1} Se + 0.5 mg l^{-1} NAA. It could be concluded that nano-Se could enhance the survival percentage of *Phalaenopsis* orchid when they applied to culture half MS medium at rooting stage at its all studied concentrations with or without NAA. On the other hand, the highest concentration of Se (10 mg l^{-1}) causing decrease in survival percentage which well improved by adding 0.5 mg l^{-1} NAA, maybe due to the toxic effect of that high level of mineral Se if it applied to culture medium alone.

TABLE 3. *In vitro* rooting of *Phalaenopsis* orchid on half MS medium with/without NAA and supplemented with different concentrations of nano-selenium

1/2 MS + Nano-Se (mg l^{-1})	1/2 MS + NAA (mg l^{-1})	Rooting (%)	Root length (cm)
0	0	88.3 c	5.2 b
10	0	100 a	6.1 ab
20	0	90 b	5.5 b
30	0	100 a	4.0 c
40	0	100 a	7.1 a
50	0	100 a	5.3 b
0	0.5	100 a	8.4 a
10	0.5	100 a	8.0 a
20	0.5	100 a	8.2 a
30	0.5	100 a	6.5 ab
40	0.5	100 a	7.7 a
50	0.5	100 a	8.5 a
F-test		*	*

Different letters in same column show significant differences among treatments according to Duncan's test at $p \leq 0.05$, NS: non-significant differences, NAA: α-napthalene acetic acid

Fig. 1. Effect of half MS medium supplemented with different concentrations of nano selenium with or without NAA on *in vitro* rooting of *phalaenopsis* orchid cv. Spring Dancer White. Scale bar = 2cm

Fig. 2. Effect of half MS medium supplemented with different concentrations of selenium with or without NAA on *in vitro* rooting of *phalaenopsis* orchid cv. Spring Dancer White. Scale bar = 2cm

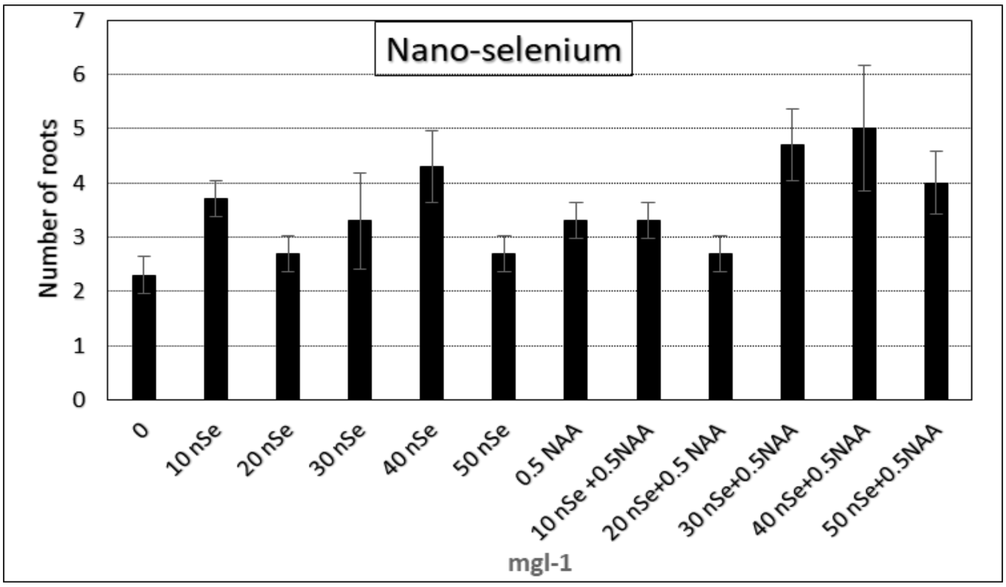

Fig. 3. Effect of half MS medium supplemented with different concentrations of nano-selenium with or without NAA on number of roots of *phalaenopsis* orchid cv. Spring Dancer White

TABLE 4. ***In vitro* rooting of *Phalaenopsis* orchid on half MS medium with/without NAA and supplemented with different concentrations of selenium**

1/2 MS + Bulk-Se (mg l^{-1})	NAA (mg l^{-1})	Rooting (%)	Root length(cm)
0	0	86.6 d	5.7 ab
0.5	0	100 a	4.7 b
1	0	100 a	6.2 a
2	0	100 a	7.0 a
4	0	100 a	3.5 bc
6	0	100 a	2.5 c
8	0	100 a	2.3 c
10	0	100 a	2.3 c
0	0.5	95.0 b	3.0 bc
0.5	0.5	100 a	4.3 b
1	0.5	100 a	6.3 a
2	0.5	100 a	5.3 ab
4	0.5	100 a	3.8 b
6	0.5	100 a	3.3 b
8	0.5	100 a	3.0 bc
10	0.5	90.7 c	2.7 c
F-test		**	**

Different letters in same column show significant differences among treatments according to Duncan's test at p ≤ 0.05, NS: non-significant differences, NAA: α-napthalene acetic acid.

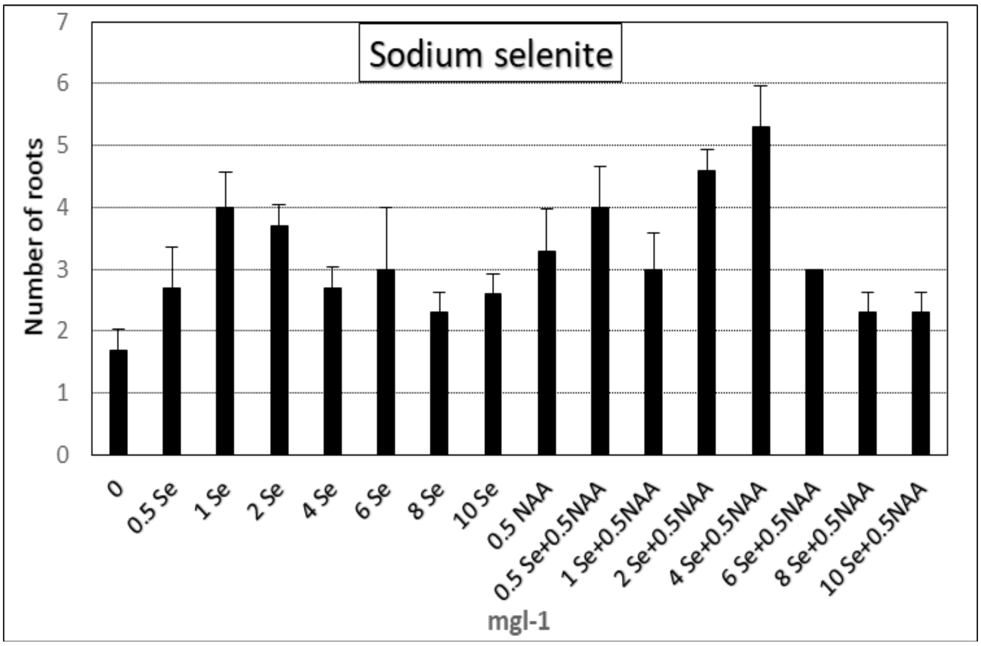

Fig. 4. Effect of half MS medium supplemented with different concentrations of selenium with or without NAA on number of roots of *phalaenopsis* orchid cv. Spring Dancer White.

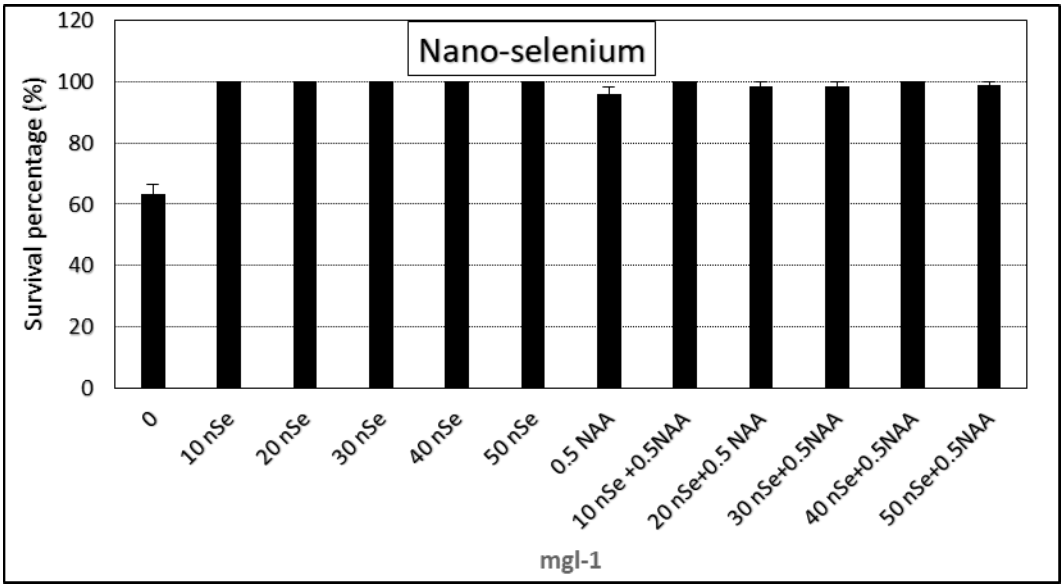

Fig. 5. Effect of half MS medium fortified with different concentrations of nano-selenium with or without NAA on survival percentage of *phalaenopsis* orchid cv. Spring Dancer White

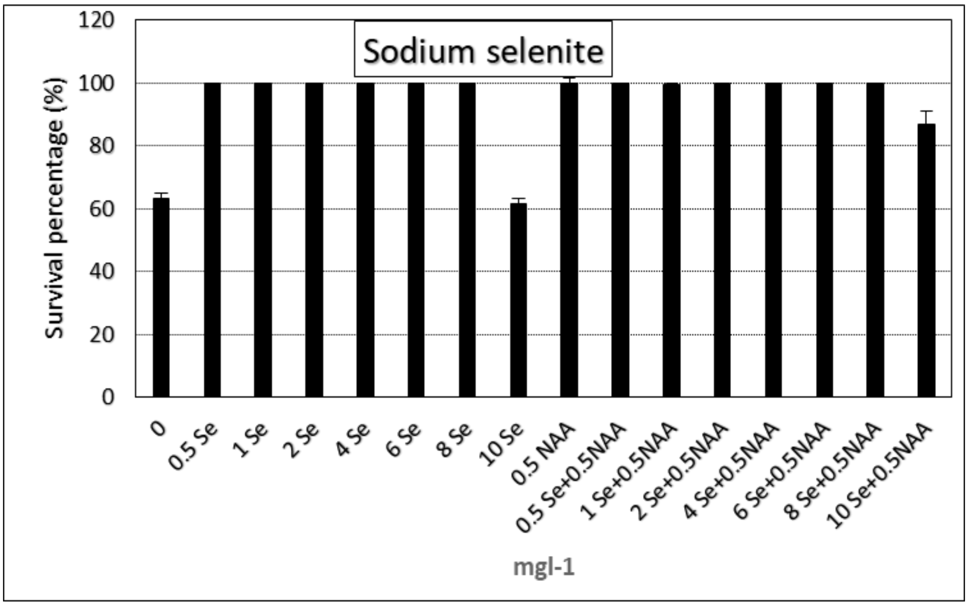

Fig. 6. Effect of half MS medium fortified with different concentrations of selenium with or without NAA on survival percentage of *phalaenopsis* orchid cv. Spring Dancer White

Discussion

The family of Orchidaceae is the second largest family among the flowering plants which their wild and cultivated flowering plants have been used in ornamental purposes, perfumery, food and traditional medicine (Kui et al. 2017). Orchid plants have very small seeds, which it is difficult to germinate in the nature because it does not contain endosperm (Koene et al. 2019). The rooting of orchid plants is considered the main obstacle that retard its propagation as reported by several researchers, who described this problem from different sides aiming to improve *in vitro* rooting and acclimatization of *Cattleya* orchid (Dewir et al. 2015), propagate endangered epiphytic orchid of *Gastrochilus matsuran in vitro* (Kang et al. 2020) and investigate the role of silicon on growth of orchid (Mantovani et al. 2018, 2020).

Plant tissue culture techniques have already contributed more than 70% of the global orchid production. Despite of the high economic value of orchid plants as one of the most important cut-flower worldwide. Unfortunately, their distinguished problem of rooting difficulty hinders its propagation and production. So, the current study was set up for more investigations on this problem by applying different forms and concentrations of Se to half MS medium with/without NAA. The selected concentrations of selenium (sodium selenite or biological nano selenium) depended on the previous studies and they were added separately to the growth medium, to improve rooting and acclimatization of orchid plants.

Tissue culture technique has become one of the most efficient methods for plants propagation particularly the hard-rooting plants like orchids. Also, this technique can produce a large number of phyto-sanitarily and physiologically high-quality, genetically similar plantlets within a limited time period. The *in vitro* propagated orchid should have a high survival percent associated with a high quality of acclimatized plantlets at the academic and commercial level (Teixeira da Silva et al. 2017). It is worth to mention that, the rooting stimulating substances have a vital role in the micropropagation process. However, no certain supporting material for rooting is optimal for all plants because the plant variety or cultivar has a wide variability in its physicochemical properties and requirements (Hoang et al. 2020). So, this current study supposes that selenium or nano-selenium could be used to improve the rooting of *Phalaenopsis* orchid *in vitro* and consequence lead to successful acclimatization process producing strong orchid transplants.

The natural propagation of orchid plants might frequently hinder due to the insignificant seed germination and its slow growth. Hence, the micropropagation is considered a viable alternative method for large-scale multiplication and conservation of orchid plants. It is worth mention that the mass propagation of orchids could be achieved *via* many *in vitro* techniques such as multiple shoot induction and somatic embryogenesis, adventitious shoot regeneration, extensive large-scale propagation (Kang et al. 2020). However, to the best of our knowledge, no information is available on the *in vitro* propagation of *Phalaenopsis* orchid under supplementation of MS medium with different concentrations of nano-Se and inorganic Se to overcome the problem of rooting and acclimatization of orchid plants.

Nanotechnology is a promising field creating innovative nanomaterials, which have a wide range of applications in different fields including agricultural, medicinal and pharmaceutical industries. The role of nanoparticles *in vitro* might include inducing the growth of cultivated plants in selected growth media (Ha et al. 2020; Khan et al. 2020 and Sotoodehnia-Korani et al. 2020), increasing the plantlets resistance or tolerance to stress like drought stress (Mozafari et al. 2018), ameliorating the oxidative damage (Pinto et al. 2019), but the rooting of plantlets still needs more investigations. No doubt that the applied nanoparticles may cause harmful impact compared to their bulk counterpart as published in case of nickel oxide nanoparticles (Pinto et al. 2019), but the biological synthesis of these particles in general might to be less toxicity and safe for the media as reported for nano-Se (Domokos-Szabolcsy et al. 2012 and 2014).

The biological role of inorganic Se and nano-Se under different growth conditions was reviewed by several researchers; hydroponics (Li et al. 2020) on garlic, field experiments (Golubkina et al. 2017) on spinach, pot experiments (Hawrylak-Nowak et al. 2018), micro-farm system (El-Ramady et al. 2015), *in vitro* (Zsiros et al. 2019) on tobacco; other studies (El-Ramady et al. (2016, 2020b), Hasanuzzaman et al. (2020) and Seliem et al. (2020). A few publications reviewed the promising impact of nano-Se on *in vitro* plants; *Nicotinia tabacum* (Domokos-Szabolcsy et al. 2012), *Arundo donax* (Domokos-Szabolcsy et al. 2014) *Capsicum annuum* (Sotoodehnia-Korani et al. 2020). This study aimed to develop a procedure for the micropropagation of *Phalaenopsis* orchid using inorganic Se and nano-Se to enhance the orchid rooting and hence its acclimatization.

The rooting process in horticulture plants might has problems, which lead to losses every year about 10–25% in nursery crops and 5% in ornamental crops worldwide. For getting desirable plant rooting, some hormones should be applied for the media or to root cuttings to enhance this processing. This previous method did not apply for all cases of orchid rooting, which needs a special handling. The use of nanomaterials in enhancing the plant rooting and its growth under *in vitro* conditions has become recently a promising tool such as silver nanoparticles (Thangavelu et al. 2018; Saha and Gupta 2018; Jadczak et al. 2019; Ha et al. 2020; Tymoszuk and Kulus 2020), iron nanoparticles (Mozafari et al. 2018 and Khan et al. 2020), zinc oxide nanoparticles (Nalci et al. 2019; Abdel Wahab et al. 2020; Mazaheri-Tirani and Dayani 2020), cobalt nanoparticles (Ha et al. 2020), gold nanoparticle (Jadczak et al. 2019), copper oxide nanoparticles (Javed et al. 2017 and Nalci et al. 2019), copper nanoparticles (Ibrahim et al. 2019), nickel oxide nanoparticles (Pinto et al. 2019), and nano-Se (Sotoodehnia-Korani et al. 2020).

The role of bulk-Se and biological nano-Se for enhancing the rooting and acclimatization of *Phalaenopsis* orchid was investigated in the current study. The low doses of Se (up to 6 mg l^{-1}) displayed growth-promoting effects, whereas the higher levels of Se at 8 and 10 mg l^{-1} were associated with toxicity and abnormality in leaf (appeared as yellowing of leaves) and malfunction of root development.The role of low added concentration of Se in promoting the *in vitro* growth was confirmed by previous studies such as stimulating the organogenesis and the growth of root system significantly by 40% of tobacco (Domokos-Szabolcsy et al. 2012), influencer for the growth-promoting of pepper (Sotoodehnia-Korani et al. 2020). The reason of these findings might be derived from the nature of two forms of Se. The selenite ion could uptake into plant tissues causing a prooxidant and excess damage directly and/or indirectly for growth and regeneration of explants. Concerning nano-Se, at low concentrations (up to 50 mg l^{-1} in current study) could generate specific physiological effects such as stimulation of organogenesis, root growth in *Phalaenopsis* orchid cultures. This study might emphasize on selenium like other nutrients could show differences in plant biological effects depending on their ionic (bulk form) and/or other forms including nano-nutrient.

Concerning the nano-Se in recent study, there is no recorded toxicity symptoms up to 50 mg l^{-1} for *in vitro* shooting and rooting of *Phalaenopsis* orchid. These findings may be confirmed by getting the highest value of rooting parameters by supplementation of the medium with 50 mg l^{-1} which stimulated in presence of NAA. The biological nano-Se might have the ability to promote the orchid growth through increasing the activities of peroxidase and catalase enzymes. Besides, the nano-Se may increase the efficient of plant roots to uptake of nutrients, which reflects on the plant growth, rooting and acclimatization.

Conclusion

Orchid plants are extremely important from the economic point of view where they share in the global market of flowers as the second remarkable plant family producing the cut-flowers. The essential method which can be used for propagation of orchid plants is *in vitro* culture technique, which share with about 70% from the total commercial production of orchid plants but orchids have a vital problem representing in the hard rooting of *in vitro* plantlets. Therefore, the recent study, the bulk Se and nano-Se were investigated on *Phalaenopsis* orchid *in vitro*, as a first report; through augmentation of MS medium with different concentrations form each of Se or nano-Se alone with and without NAA. As recorded from our previous *in vitro* experiments on nano-Se and inorganic Se (selenite), the used applied nano-Se dose (up to 50 mg l^{-1}) did not cause any toxicity symptoms on plantlets, whereas the bulk Se or selenite at higher concentrations (8 and 10 mg l^{-1}) showed distinguished toxicity symptoms. In the current study, the most important findings confirmed that supplementation of MS media with Se (up to 6 mg l^{-1}) and nano-Se (up to 50 mg l^{-1}) in presence of NAA could be enhance rooting and acclimatization of *Phalaenopsis* orchid without significant effect on shoot multiplication. This is preliminary study so, further *in vitro* investigations on orchids are required to involve a wide range of nano-Se concentrations and up to more than 50 mg l^{-1} and examine the effect of other more nanoparticles alone and in combination with nano-Se on physiological, biochemical, genetical and anatomical aspects.

Author Contributions

This study was designed and implemented by all authors. They contributed in writing the paper, interpreting information presented and they all have read and agreed to the version of the manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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