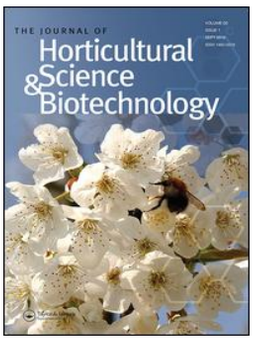

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Nitric oxide in combination with indole-3-butyric acid improves root growth in 'Ferdor Julior' hardwood cuttings (*Prunus insititia* (L.) × *Prunus domestica* (L.))

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ABSTRACT

The production of plants for high density fruit orchards requires a highly efficient clonal propagation method. 'Ferdor Julior' rootstock is well adapted to different soil conditions, but its natural rooting potential is very limited. Propagation treatments, such as bottom heat and indole-3-butyric acid (IBA) applications have previously been used to improve rooting. The aim of this work is to evaluate the effects of either IBA, or sodium nitroprusside (SNP; a nitric oxide (NO) donor), or a combination of both on the rooting of hardwood cuttings of 'Ferdor Julior'. As metabolism markers, ascorbic acid (AA) and glutathione (GSH) were measured in adventitious roots. 'Ferdor Julior' hardwood cuttings were treated as follows: untreated, 0.3 mM IBA, 1 mM SNP, 1 mM SNP previously exposed to light (as a negative control), and 1 mM SNP + 0.3 mM IBA. After 4 months, the combined use of SNP and IBA had increased root growth and lateral rooting, but resulted in less shoot growth, whilst AA and GSH concentrations were also reduced. IBA and SNP individual treatments showed intermediate results, compared to untreated cuttings. According to the results obtained, there may be an additive effect of auxin and NO signaling pathways. In conclusion, this new and promising technique for 'Ferdor Julior' propagation could improve lateral root development and promote early lateral root growth.

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KEYWORDS
Plant propagation; auxin; *Prunus* spp. rootstock; lateral roots; ascorbic acid; glutathione

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Introduction

The root is an important organ for plants. It is involved in many functions, such as anchorage, nutrition and water acquisition, nutrient storage, biosynthesis of secondary metabolites, and propagation (de Dorlodot et al., 2007). Apart from other functions, such as embryogenesis, phototropism, gravitropism, shoot dominance, and leaf, flower, vascular and fruit development, auxin governs root growth and differentiation. Auxins are applied to hardwood cuttings to enhance the success of clonal propagation (Ljung, 2013, and references therein). Within this hormone group, indole-3-acetic acid (IAA) is the most abundant endogenous auxin (Davies, 2010), while indole-3-butyric acid (IBA) is less frequent. IBA is a precursor of IAA by β-oxidation, and contributes to the transport of auxin through the plant (Strader & Bartel, 2011). IBA is a more stable form of auxin, and is more commonly used exogenously to enhance the strike rate of cuttings (Skůpa, Opatrný, & Petrášek, 2014, and references therein). Primary root, lateral root, and root hair formation are cross-regulated by many hormonal interactions (Saini, Sharma, Kaur, & Pati, 2013). The majority of auxin forms (especially IAA) in plant tissues are conjugated, such as derived sugars, peptides, amino acids, or myo-inositol, via ester or amide linkages (Normanly, Slovin, & Cohen, 1995). The purpose of this auxin conjugation has been related to storage, transport, compartmentalisation, auxin detoxification and protection against degradation (Cohen & Bandurski, 1982).

Nitric oxide (NO) is a signaling molecule, widely studied in animals (Wink & Mitchell, 1998), and later discovered in plants (Lamattina, García-Mata, Graziano, & Pagnussat, 2003, and references therein). The first report showed that herbicide-treated soybean plants can release NO (Klepper, 1979). Some years later, research was focused on the phytotoxic effects of different nitrogen oxides upon vegetation, including NO (Rowland, Murray, & Welburn, 1985). NO is associated with plant growth processes, development, photomorphogenic responses, leaf and root growth (Lamattina et al., 2003), shoots and pollinic tube growth (Salmi, Morris, Roux, & Porterfield, 2007), and senescence (Beligni & Lamattina, 2000; Leshem & Pinchasov, 2000; Leshem, Wills, & Ku, 1998). Its synthesis has also been associated with

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biotic and abiotic stress (Lamattina et al., 2003, and references therein).

In root growth and development, NO studies have been focused on the biosynthetic precursor (Flores et al., 2008) and root hair formation (Lombardo, Graziano, Polacco, & Lamattina, 2006) in *Arabidopsis thaliana*, gravitropism bending coupled to auxin induction in *Glycine max* (Hu, Neill, Tang, & Cai, 2005), lateral rooting in *Solanum lycopersicum* (Correa-Aragunde, Graziano, & Lamattina, 2004; Guo, Xia, & Yang, 2008) and *Oryza sativa* (Chen, Chao, Hsu, Hong, & Kao, 2012), and adventitious rooting in *Cucumis sativus* (Pagnussat, Lanteri, Lombardo, & Lamattina, 2004).

No investigation has been carried out into the effects of the combination of auxin and NO on the rooting of *Prunus*spp. hardwood cuttings. There is already a known relationship between NO and auxin, as has been proved by the application of a NO donor, such as sodium nitroprusside (SNP), which mimics the effect of auxin (Chen et al., 2012). It has recently been observed that there is a crosstalk between NO and auxin in root promotion of *Eucalyptus*spp. cuttings (Abu-Abied et al., 2012). These results confirm that there is a stimulation of root growth, and adventitious root development, by NO in evergreen woody perennials (Correa-Aragunde et al., 2007; Fernandez-Marcos, Sanz, Lewis, Muday, & Lorenzo, 2011); however, the effects of NO on rooting of deciduous *Prunus*spp. is presently unknown, to the best of our knowledge.

Although the mechanisms of root growth from seedlings are different from those found in adventitious root formation, there are some coincidences between the effects of NO observed in the present work, and its effects on herbaceous species. Pagnussat, Lanteri, and Lamattina (2003) showed that NO is necessary for adventitious root formation in seedlings. The only reference we have found concerning rooting and NO in evergreen hardwood cuttings is the work done by Abu-Abied et al. (2012). The effects on root development have never been tested on hardwood cuttings of *Prunus*spp. rootstocks.

In actively growing organs, such as meristems, certain kinds of metabolites are necessary for cell division and expansion, including ascorbic acid (AA) and its redox partner, glutathione (GSH) (Smirnoff, 2000). There is experimental evidence that AA accelerates cell division in *Allium*spp., *Pisum*spp., and *Lupinus*spp. roots (Arrigoni, Calíbrese, De Gara, Bitonti, & Liso, 1997; Citterio, Sgorbati, Scippa, & Sparvoli, 1994; de Cabo, González-Reyes, Córdoba, & Navas, 1996) by increasing the number of cells that pass from the G1 stage (quiescent) to the S stage (transcriptional) (Davey et al., 2000; Navas & Gomez-Diaz, 1995). Similar results have been found in the apex, when AA has been applied, and the growth effect was governed by the redox state of this antioxidant (de Pinto, Francis, & De Gara, 1999), which promoted dormancy or, conversely, renewed activity during the growing season (Gergoff Grozeff & Bartoli, 2013). Furthermore, low AA synthesis mutants of *Arabidopsis thaliana*, such as *vtc2*, present more lateral roots than wild plants (Olmos, Kiddle, Pellny, Kumar, & Foyer, 2006). AA and its redox state also interact with auxins (Lee, Kim, & Kim, 2007) and determine gravitropic response in roots (Lee et al., 2011).

A great number of studies have addressed the effects of AA on the development of many plant organs and tissues, as a direct or indirect consequence of the activity of the enzyme ascorbic acid oxidase (Davey et al., 2000). AA and its redox state also govern other important processes in roots, such as gravitropism (Lee et al., 2011) or cell division (Davey et al., 2000).

According to previous work by Dessy, Radice, & Andorno (2004), *Prunus*spp. rootstocks, such as 'Ferdor Julior', are difficult to propagate by cuttings. Therefore, the aim of this work is to evaluate the different possible effects of applications of a NO donor (such as SNP) individually, or IBA individually, or a combination of both, on root initiation and root growth of 'Ferdor Julior' rootstock cuttings during the first stages of rooting and, at the same time, to control the redox state of AA and GSH as markers of active cell metabolism.

Materials and methods

Plant material and treatments

'Ferdor Julior' hardwood cuttings (*Prunus insistitia* (L.) × *Prunus domestica* (L.)) were collected in winter from stock mother plants, cultured since 1999, and growing in field soil at the Experimental Station 'Julio A. Hirschhorn' of La Plata, Argentina (National University of La Plata) (34°55′S, 57°57′W). Cuttings were 20 cm long and 1.5 cm in diameter, on average, and were collected in July 2013. The hardwood cuttings had 10 buds each, on average. Cuttings were immediately taken to the laboratory, and the treatments were performed in two steps. First, one group of 200 hardwood cuttings was dipped in water, another group of 200 in 1 mM water solution of SNP, and a third group of 100 in 1 mM water solution of SNP previously exposed to light (SNPi), in order to release the NO from the solution. The SNPi solution was prepared in the same way as the SNP solution, but the SNPi was exposed to light (100 µmol m^{-2} s^{-1}) for 24 h in order to release NO and leave cyanide in the solution. To assess SNP effects, both photodegraded SNP and the SNP analogue Na_4[Fe

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$(CN)_6$] (SNPi) were used, in accordance with Jasid, Simontacchi, & Puntarulo (2008), so as to ensure that any effects were caused by the presence of NO only, and not by the cyanide released by the solubilisation of SNP. The concentration of SNP was taken from previous work, performed by Jasid et al. (2008). These three treatments were performed for 12 h to ensure maximum exposure of the cuttings to NO. Then 100 cuttings treated with water, and another 100 cuttings treated with SNP, were immersed in new solutions of 0.3 mM IBA for another extra 12 h to carry out the combination treatments of IBA and SNP + IBA, respectively. The concentration of IBA was taken from a previous study (Dessy et al., 2004). Ten cuttings from each treatment were then cultured in perlite:soil mixture, in 1 L pots placed in a greenhouse at the Centro de Propagación Vegetativa (CIC PBA La Plata – UNLP) from 1st September till the end of December 2013. Pots were randomly distributed in the greenhouse to avoid possible differences in light distribution. One hundred hardwood cuttings were used in each treatment. The sequence of all the treatments can be seen in Supplementary Figure 1. Temperature was measured in the greenhouse every half an hour during the growing season, at 1.0 m height, and it reached an average temperature of 19.4°C in September, 21.5°C in October, 23.1°C in November, and 25.9°C in December. Temperature data was recorded by a Licor LI-1400 data logger (equipped with a 1400–101 air temperature sensor). Growth determinations and samples were collected two and four months after plantation. Samples from roots were harvested, weighed, immediately frozen in liquid nitrogen, and stored at −80°C until use. Ten replicates were taken for each treatment and harvest time. The three experiments, with the same treatments, were performed simultaneously in the same greenhouse.

Rooting and shoot and root growth

Cuttings were cultured for four months, and evaluations of shoot growth were performed monthly, as described in Dessy et al. (2004). The percentage of rooting was determined during early establishment of the cuttings, two months after plantation. Samples were collected two and four months after plantation. Shoot length was determined from the upper bud to the bottom of the growing annual shoot. Plants were then carefully taken out of the pots, roots separated from the soil mixture, and cleaned with tap water. Roots were dried with paper towel and total fresh weight was measured. Then, roots were divided into two groups, according to the criterion used by Graciano, Tambussi, Castán, & Guiamet (2009): primary roots (>1 mm diameter) and lateral roots (<1 mm diameter). Root weight and number of lateral roots were determined in 10 plants, in triplicate.

Determination of AA and GSH, and their corresponding redox states

Two hundred mg of the root samples that had been frozen in liquid nitrogen and stored at −80°C were processed in a mortar and pestle, with the addition of liquid nitrogen, to obtain a frozen powder, then 1 ml of 3% v/v trifluoroacetic acid solution (kept in an ice-bath) was added and the samples were processed a second time to release the antioxidants from the samples. The samples were centrifuged at 13,000 g for 10 min, and the supernatant was used for monitoring AA, GSH, and the redox state of both antioxidants. All the extraction steps of the samples were carefully kept in ice, or below 4°C, to avoid antioxidant degradation and/or oxidation. Five hundred µL of the supernatant were placed in a solid phase extraction column C18 (Bond Elut C18, Agilent Technologies, Santa Clara, United States). Five hundred µL of a potassium phosphate buffer, pH = 7, were added to set the sample inside the column. Next, 1.5 mL were captured in an Eppendorf tube and used for the determinations. The efficiency of the extraction technique was checked with L-Ascorbic Acid pure standard (Sigma-Aldrich®). AA and dehydroascorbate (DHA) were determined by high-performance liquid chromatography, according to the method of Bartoli et al. (2006), and GSH and glutathione disulfide (GSSG) were measured by spectrophotometer, following Griffith (1980). Total AA content was measured after reducing DHA with 1 mM dithiothreitol. The redox state of AA was calculated as a percentage of DHA = [(Total content − AA content)/Total AA content^{-1}] × 100, and the redox state of GSH was calculated as a percentage of GSSG = [Total GSH − GSH content)$^{-1}$/ Total GSH)] × 100. The final results represent the amount of these antioxidants present in the tissue, expressed in mmol gr^{-1} fresh weight.

Statistical analysis

Data presented in this study as means are the averaged results obtained from three independent and simultaneous experiments, containing 10 plants each, and recording rooting percentage, shoot growth, root weight, and number of secondary roots. In the figures, vertical bars represent standard deviation. In order to measure antioxidant concentrations and the respective redox state, roots were chosen randomly from three plants representing each of the three replicates. Experimental layout was performed in a completely randomised design.

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Data were analysed by means of one-way ANOVA. The means were compared with the least significant difference test, at a significance level of 0.05. Statistical analysis was performed with Statistica® 6.0 software from StatSoft.

Results and discussion

Rooting and shoot and root growth

In response to the application of SNP (87.50%), IBA (96.88%), SNPi (90.63%), and the combination of SNP + IBA (93.75%), rooting was improved, showing no statistical differences among treated hardwood cuttings, whilst untreated cuttings reached only 59.38% (Figure 1). 'Ferdor Julior' hardwood cuttings have low natural capacity to produce adventitious roots, but they can be successfully multiplied by micropropagation (Radice, Perelman, & Caso, 1999). Further studies, carried out by Dessy et al. (2004), demonstrated that the application of low doses of IBA (1% w/v, equivalent to 0.3 mM) improved the rooting percentage in 'Ferdor Julior' hardwood cuttings, showing similar effects on other *Prunus* rootstocks. The effect of IBA was even improved when hardwood cuttings were taken from the proximal portion of the hardwood shoots, and bottom heat (21 ± 2°C) was applied during the rooting process (Dessy et al., 2004). In the present study, the combination of IBA + SNP, or SNP individually, showed a similar effect on the rooting percentage, compared to IBA treatment alone (Figure 1). The effect of NO donors, such as SNP, combined with IBA, was also reported in rice by Chen et al. (2012). These authors found that NO and auxin stimulated lateral rooting, while the application of a NO scavenger (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide) produced the opposite effect.

During the growing season, primary shoot height increased throughout the experiment, without any differences being detected among the untreated, SNP, IBA, or SNPi treatments; however, in contrast to the rest of the treatments, combined treatments of SNP + IBA showed less shoot height from November to December, with high statistical differences (Figure 2).

The effect on root weight was enhanced by the application of SNP + IBA, or IBA individually, after two months of plantation, but these differences were not significant after four months (Figure 3). Four months after plantation, all of the treated cuttings showed differences, compared to the untreated ones, in shoot height (Figure 2) and root fresh weight (Figure 3). The combined treatment of SNP + IBA showed no difference on root fresh weight, compared to the cuttings treated with IBA individually, but there was a slight difference between the root weight of cuttings treated with SNP and SNPi, when compared with untreated cuttings (Figure 3). The combined treatment of SNP and IBA showed a clear effect on the development of secondary roots from an early stage of development, keeping this difference 4 months after plantation (Figure 4). SNP might help root development, together with IBA, in the early stages of hardwood plantation. Similar results were found in *Arabidopsis thaliana* by Flores et al. (2008), where NO acted as a signal in root development, especially in lateral seedling roots, coupled with the effect of auxin. SNP-treated cuttings showed no difference in lateral root number, compared to the untreated cuttings, while intermediate results were found in the IBA and SNPi treatments, two months after plantation (Figure 4). Four months after plantation, differences were still significant, showing a good number of lateral roots in the combined treatment (SNP + IBA), followed by the SNPi-treated cuttings

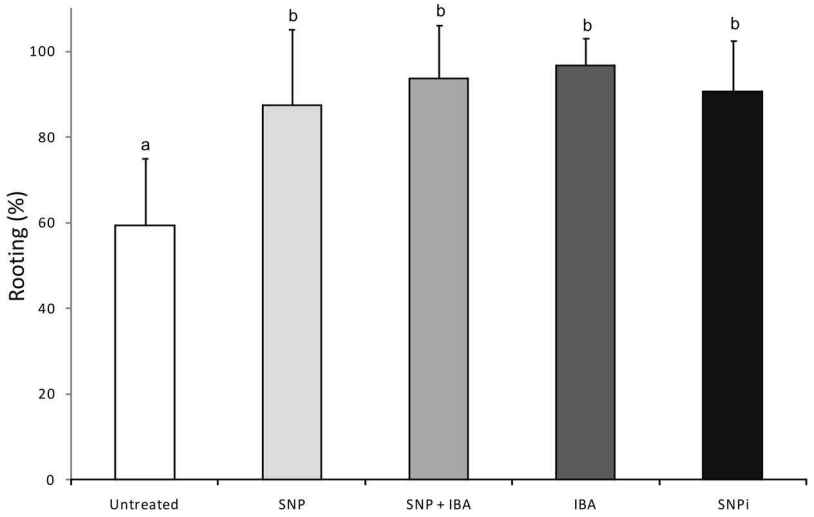

Figure 1. Percentage of rooting of untreated, 1 mM SNP, 1 mM SNP + 0.3 mM IBA, 0.3 mM IBA, and 1 mM SNPi treated 'Ferdor Julior' hardwood cuttings at two months after plantation. Vertical bars show the standard deviation. Different letters denote significant statistical differences between treatments (ANOVA, $p \leq 0.05$).

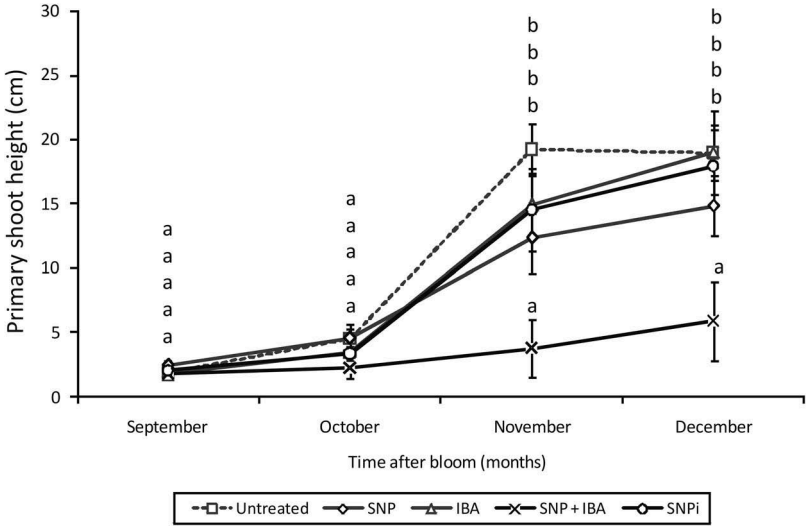

Figure 2. Primary shoot height of untreated, 1 mM SNP, 0.3 mM IBA, 1 mM SNP + 0.3 mM IBA, and 1 mM SNPi treated 'Ferdor Julior' hardwood cuttings over four month interval. Vertical bars show the standard deviation. Different letters denote significant statistical differences between treatments (ANOVA, $p \leq 0.05$).

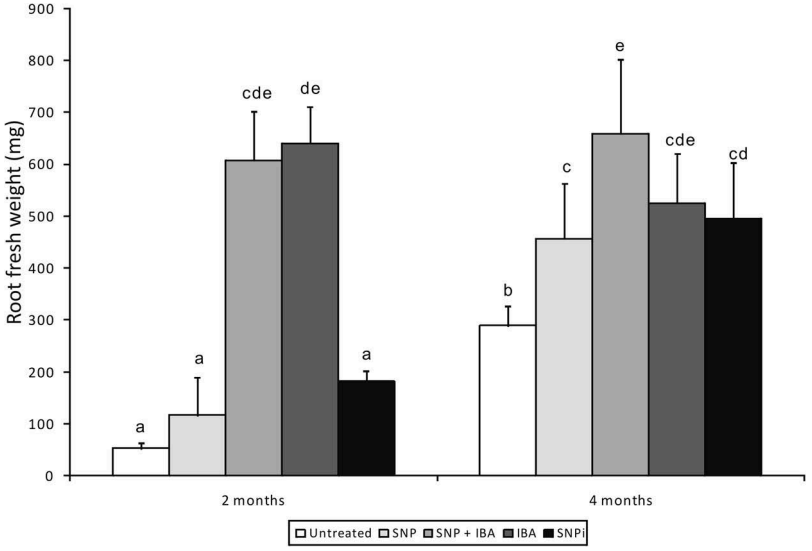

Figure 3. Root fresh weight of untreated, 1 mM SNP, 0.3 mM IBA, 1 mM SNP + 0.3 mM IBA and 1 mM SNPi treated 'Ferdor Julior' hardwood cuttings at two and four months after plantation. Vertical bars show the standard deviation. Different letters denote significant statistical differences between treatments (ANOVA, $p \leq 0.05$).

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(Figure 4). Jasid et al. (2008) found that sorghum embryonic axes, from seeds treated with SNPi and untreated control seeds, exhibited no difference in germination percentage, neither on dry nor on fresh weight of the plants, during germination. Further research in the mechanisms of rooting, root growth, and lateral root generation is needed to prove this side effect of SNPi on hardwood cuttings of 'Ferdor Julior' (Figure 4).

There are no examples of root formation in fruit hardwood cuttings related to the effects of NO. Most of the experiments performed with NO have been developed with herbaceous species, such as *Solanum lycopersicum* (Correa-Aragunde et al., 2004; Guo et al., 2008), *Cucumis sativus* (Pagnussat et al., 2003), *Sorghum*spp. seedlings (Jasid et al., 2008), and *Oryza sativa* plants (Chen et al., 2012). Exceptionally, there is an example of rooting treatments with NO and auxin of *Eucalyptus grandis* hardwood cuttings (Abu-Abied et al., 2012). As mentioned in the introduction, the mechanisms of root growth in hardwood cuttings and seedlings are different, but the effect of NO in herbaceous species shows that NO is necessary for root growth (Jasid et al., 2008) and adventitious rooting (Pagnussat et al., 2003). In accordance with our findings, the same effect was observed in *Cucumis sativus* roots, when auxin and NO are combined (Pagnussat et al., 2003). This effect on root development has never been tested before on deciduous hardwood cuttings, nor on *Prunus*spp. rootstocks.

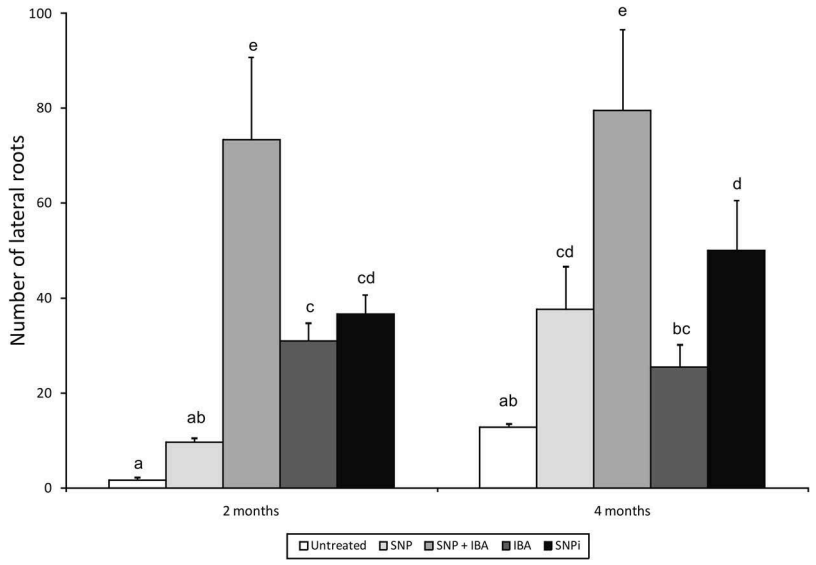

Figure 4. Number of lateral roots of untreated, 1 mM SNP, 0.3 mM IBA, 1 mM SNP + 0.3 mM IBA, and 1 mM SNPi treated 'Ferdor Julior' hardwood cuttings at two and four months after plantation. Vertical bars show the standard deviation. Different letters denote significant statistical differences between treatments (ANOVA, $p \leq 0.05$).

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Ascorbic acid and glutathione content, and their respective oxidised forms

Two months after plantation, no difference was found in the concentration of AA in any of the cuttings treated, when compared to the untreated ones (Figure 5(a)), while a reduction of the oxidised form of AA was observed in cuttings treated with SNP, when compared to the untreated ones. SNP, SNP + IBA, and SNPi showed an increase in the redox state of over 60% (Figure 5(b)); however, when cuttings were growing, four months after plantation, there was a decrease in the oxidised form of AA in the SNP-, SNP + IBA-, and SNPi-treated cuttings, compared to the samples taken two months before. Untreated and SNP-treated cuttings showed no differences in the redox state, when compared to the cuttings collected after two months of the same treatments (Figure 5(b)).

The concentration of GSH was no different in two-month-old untreated or treated cuttings, while it clearly decreased in the untreated, and cuttings treated with SNP+IBA, four months after plantation, when compared to the rest of the treatments (Figure 6(a)). The effect of GSH partially mimics the effect found in AA (Figure 4(a)). When the redox state was measured, no differences were found in the percentage of GSSG in two-month-old roots, and a rise in the oxidised form of GSH was observed in the untreated, and in the SNP-treated cuttings, when compared to the initial evaluation (Figure 6(b)).

The concentrations of AA (Figure 5(a)) and GSH (Figure 6(a)) decreased in roots treated with SNP + IBA. Similarly, in actively growing roots, the demand for antioxidants is high, producing a decrease in the amount of antioxidants (Xu, Zhu, Chen, Gong, & Liu, 2013). The same effect was found in two AA-deficient mutants, *Arabidopsis thaliana* (*vtc1/vtc2* mutants), wherein Lee et al. (2011) observed a great number of lateral roots, compared to the wild type. Similar results were found by Olmos et al. (2006) in *Arabidopsis thaliana vtc2* mutant plants. These results, along with those found in the present work, show an association of increased lateral root growth with lower content of AA. In the present work, this relationship is observed after the combined treatment of SNP and IBA – a decrease in the antioxidant concentration (AA – Figure 5(a), and GSH – Figure 6(a)), concomitant with a proliferation of lateral roots (Figure 4).

For *in vitro* shoot propagation of *Prunus avium* × *Prunus mahaleb* rootstock, it was found that auxin, combined with myo-inositol, can improve shoot height, but auxin alone can enhance shoot weight (Sarropoulou, Dimassi-Theriou, & Therios, 2015). These authors did not find any relationship with other biochemical parameters, such as carbohydrate, chlorophyll, nor endogenous proline leaf content in the treatments. When biochemical parameters were measured in this work, the concentrations of AA (Figure 5(a)) and GSH (Figure 6(a)) were reduced in the SNP + IBA-treated hardwood cuttings, which showed a significant proliferation of lateral roots, accompanied by a reduction of the redox state of the oxidased form of AA (Figure 5 (b)), when compared to the rest of the treatments. Rooting is a complex process that has not yet been fully understood, and therefore requires further research into other biochemical parameters to predict the rooting capacity of different propagation plant organs.

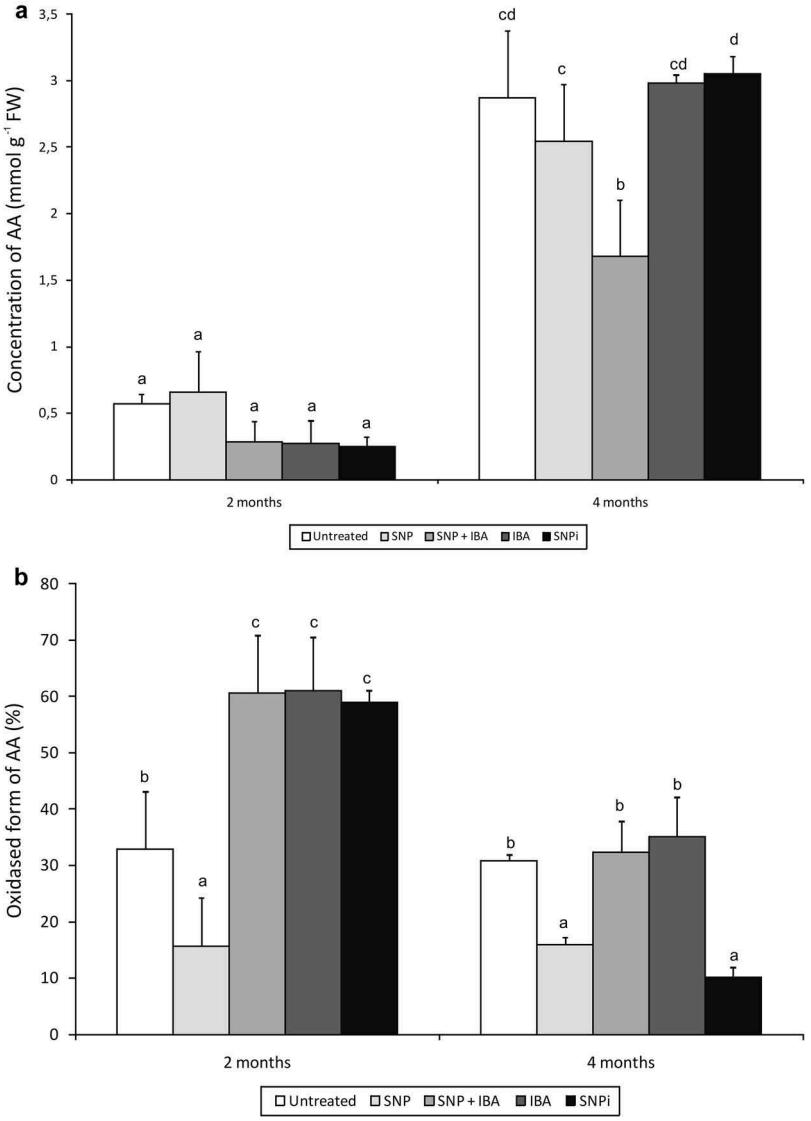

Figure 5. Concentration of AA (a), and its redox state (b), in roots of untreated, 1 mM SNP, 0.3 mM IBA, 1 mM SNP + 0.3 mM IBA, and 1 mM SNPi treated 'Ferdor Julior' hardwood cuttings at two and four months after plantation. Vertical bars show the standard deviation. Different letters denote significant statistical differences between treatments (ANOVA, $p \leq 0.05$).

In conclusion, these results show that SNP and IBA can modify shoot length, root weight, and lateral root number, making the combination studied here a tool to improve plant propagation of 'Ferdor Julior' cuttings that would probably not survive the first stage of a new plant nursery. In terms of practical application, delayed shoot development in SNP + IBA-treated cuttings could be a problem for certain kinds of grafting techniques (as described in Hartmann & Kester, 1987). Therefore, this could imply a longer growing period for the rootstock. According to these findings, further work should be done to determine how each treatment then behaves in the nursery, in the subsequent growing season. Additionally, AA and its redox state could be promising metabolic markers to predict lateral rooting in clonally propagated hardwood cuttings.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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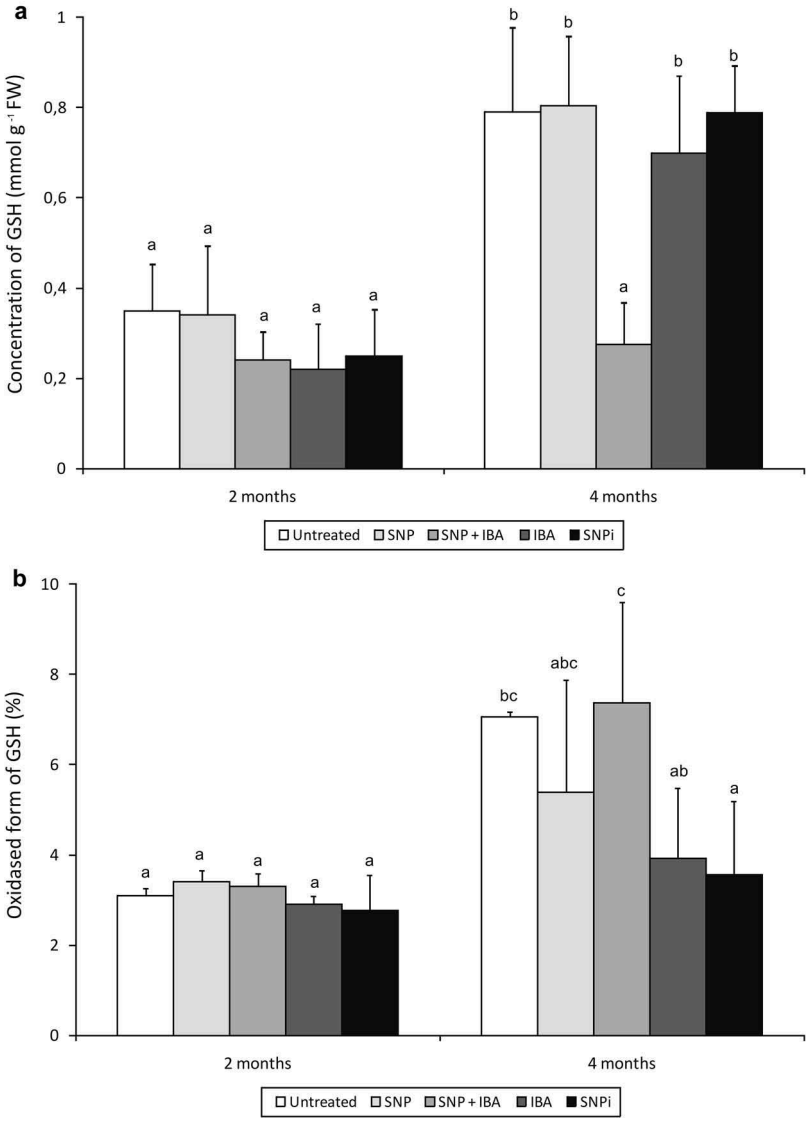

Figure 6. Concentration of GSH (a), and its redox state (b), in roots of untreated, 1 mM SNP, 0.3 mM IBA, 1 mM SNP + 0.3 mM IBA, and 1 mM SNPi treated 'Ferdor Julior' hardwood cuttings at two and four months after plantation. Vertical bars show the standard deviation. Different letters denote significant statistical differences between treatments (ANOVA, $p \leq 0.05$).

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References

Abu-Abied, M., Szwerdszarf, D., Mordehaev, I., Levy, A., Rogovoy, O., Belausov, E., … Sadot, E. (2012). Microarray analysis revealed upregulation of nitrate reductase in juvenile cuttings of *Eucalyptus grandis*, which correlated with increased nitric oxide production and adventitious root formation. *The Plant Journal*, *71*, 787–799. doi:10.1111/j.1365-313X.2012.05032.x

Arrigoni, O., Calíbrese, G., De Gara, L., Bitonti, M.B., & Liso, R. (1997). Correlation between changes in cell ascorbate and growth of *Lupinus albus* seedlings. *Journal of Plant Physiology*, *150*, 302–308. doi:10.1016/S0176-1617(97)80125-3

Bartoli, C.G., Yu, J., Gómez, F., Fernández, L., Mcintosh, L., & Foyer, C.H. (2006). Inter-relationships between light and respiration in the control of ascorbic acid synthesis and accumulation in *Arabidopsis thaliana* leaves. *Journal of Experimental Botany*, *57*, 1621–1631. doi:10.1093/jxb/erl005

Beligni, M.V., & Lamattina, L. (2000). Nitric oxide stimulates seed germination and deetiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta*, *210*, 215–221. doi:10.1007/PL00008128

Chen, Y.-H., Chao, Y.-Y., Hsu, Y.Y., Hong, C.-Y., & Kao, C.-H. (2012). Heme oxygenase is involved in nitric oxide- and auxin-induced lateral root formation in rice. *Plant Cell Reports*, *31*, 1085–1091. doi:10.1007/s00299-012-1228-x

Citterio, S., Sgorbati, S., Scippa, S., & Sparvoli, E. (1994). Ascorbic acid effect on the onset of cell proliferation in pea root. *Physiologia Plantarum*, *92*, 601–607. doi:10.1111/ppl.1994.92.issue-4

Cohen, J.D., & Bandurski, R.S. (1982). Chemistry and physiology of the bound auxins. *Annual Review of Plant Physiology*, *33*, 403–430. doi:10.1146/annurev.pp.33.060182.002155

Correa-Aragunde, N., Lanteri, M.L., García-Mata, C., Ten Have, A., Laxalt, A.M., Graziano, M., & Lamattina, L.

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(2007). Nitric oxide functions as intermediate in auxin, abscisic acid, and lipid signaling pathways. In L. Lamattina & J.C. Polacco (Eds.), *Plant cell monographs. Nitric oxide in plant growth* (pp. 113–130). Berlin, Heidelberg: Springer-Verlag.

Correa-Aragunde, N.M., Graziano, M.L., & Lamattina, L. (2004). Nitric oxide plays a central role in determining lateral root development in tomato. *Planta*, *218*, 900–905. doi:10.1007/s00425-003-1172-7

Davey, M.W., Van Montagu, M., Inzé, D., Sanmartin, M., Kanellis, A., Smirnoff, N., … Fletcher, J. (2000). Plant L-ascorbic acid: Chemistry, function, metabolism, bioavailability and effects of processing. *Journal of the Science of Food and Agriculture*, *80*, 825–860. doi:10.1002/(SICI)1097-0010(20000515)80:7<825::AID-JSFA598>3.0.CO;2-6

Davies, P.J. (2010). Plant hormones: Their nature, occurrence, and functions. In P.J. Davies (Ed.), *Plant hormones* (pp. 1–15). Dordrecht: Springer.

de Cabo, R.C., González-Reyes, J.A., Córdoba, F., & Navas, P. (1996). Rooting hastened in onions by ascorbate and ascorbate free radical. *Journal of Plant and Growth Regulation*, *15*, 53–56. doi:10.1007/BF00192931

de Dorlodot, S., Forster, B., Pagès, L., Price, A., Tuberosa, R., & Draye, X. (2007). Root system architecture: Opportunities and constraints for genetic improvement of crops. *Trends in Plant Science*, *12*, 474–481. doi:10.1016/j.tplants.2007.08.012

de Pinto, M.C., Francis, D., & De Gara, L. (1999). The redox state of the ascorbate-dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. *Protoplasma*, *209*, 90–97. doi:10.1007/BF01415704

Dessy, S., Radice, S., & Andorno, A. (2004). Ferdor-Julior, Myriam-Yumir and Julien GF 655-2 rootstocks: Propagation by cuttings with growth regulators and bottom heat. Proceedings of the 1st IS rootstocks – Deciduous fruit. Eds. M.Á. Moreno Sánchez and A.D. Webster. ISHS. *Acta Horticulturae*, *658*, 629–635. doi:10.17660/ActaHortic.2004.658.95

Fernandez-Marcos, M., Sanz, L., Lewis, D.R., Muday, G.K., & Lorenzo, O. (2011). Nitric oxide causes root apical meristem defects and growth inhibition while reducing PIN-FORMED 1(PIN1)-dependent acropetal auxin transport. *Proceedings of the National Academy of Sciences*, *108*, 18506–18511. doi:10.1073/pnas.1108644108

Flores, T., Todd, C.D., Tovar-Mendez, A., Dhanoa, P.K., Correa-Aragunde, N., Hoyos, M.E., … Polacco, J.C. (2008). Arginase-negative mutants of arabidopsis exhibit increased nitric oxide signaling in root development. *Plant Physiology*, *147*, 1936–1946. doi:10.1104/pp.108.121459

Gergoff Grozeff, G.E., & Bartoli, C.G. (2013). Participation of AA in the dormancy establishment of poplar lateral branch buds. *Journal of Forest Research*, *19*, 301–304. doi:10.1007/s10310-013-0410-5

Graciano, C., Tambussi, E.A., Castán, E., & Guiamet, J.J. (2009). Dry mass partitioning and nitrogen uptake by *Eucalyptus grandis* plants in response to localized or mixed application of phosphorus. *Plant and Soil*, *319*, 175–184. doi:10.1007/s11104-008-9859-y

Griffith, O.W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical Biochemistry*, *106*, 207–212. doi:10.1016/0003-2697(80)90139-6

Guo, K., Xia, K., & Yang, Z.-M. (2008). Regulation of tomato lateral root development by carbon monoxide and involvement in auxin and nitric oxide. *Journal of Experimental Botany*, *59*, 3443–3452. doi:10.1093/jxb/ern194

Hartmann, H.T., & Kester, D.E. (1987). Techniques of grafting. In H.T. Hartmann & D.E. Kester (Eds.), *Plant propagation: Principles and practices* (4th ed., pp. 421–470). Englewood Cliffs, NJ: Prentice-Hall.

Hu, X., Neill, S.J., Tang, Z., & Cai, W. (2005). Nitric oxide mediates gravitropic bending in soybean roots. *Plant Physiology*, *137*, 663–670. doi:10.1104/pp.104.054494

Jasid, S., Simontacchi, M., & Puntarulo, S. (2008). Exposure to nitric oxide protects against oxidative damage but increases the labile iron pool in sorghum embryonic axes. *Journal of Experimental Botany*, *59*, 3953–3962. doi:10.1093/jxb/ern235

Klepper, L. (1979). Nitric oxide (NO) and nitrogen dioxide (NO_2) emissions from herbicide-treated soybean plants. *Atmospheric Environment (1967)*, *13*, 537–542. doi:10.1016/0004-6981(79)90148-3

Lamattina, L., García-Mata, C., Graziano, M., & Pagnussat, G. (2003). Nitric oxide: The versatility of an extensive signal molecule. *Annual Review of Plant Biology*, *54*, 109–136. doi:10.1146/annurev.arplant.54.031902.134752

Lee, Y., Kim, M.W., & Kim, S.H. (2007). Cell type identity in Arabidopsis roots is altered by both ascorbic acid-induced changes in the redox environment and the resultant endogenous auxin response. *Journal of Plant Biology*, *50*, 484–489. doi:10.1007/BF03030687

Lee, Y., Park, C.H., Kim, A.R., Chang, S.C., Kim, S.-H., Lee, W.S., & Kim, S.-K. (2011). The effect of ascorbic acid and dehydroascorbic acid on the root gravitropic response in *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, *49*, 909–916. doi:10.1016/j.plaphy.2011.05.013

Leshem, Y.Y., & Pinchasov, Y. (2000). Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripening of strawberries *Fragaria anannasa* (Duch.) and avocados *Persea americana* (Mill). *Journal of Experimental Botany*, *51*, 1471–1473.

Leshem, Y.Y., Wills, R.B.H., & Ku, V.V.V. (1998). Evidence for the function of the free radical gas – Nitric oxide (NO center dot) – As an endogenous maturation and senescence regulating factor in higher plants. *Plant Physiology and Biochemistry*, *36*, 825–833. doi:10.1016/S0981-9428(99)80020-5

Ljung, K. (2013). Auxin metabolism and homeostasis during plant development. *Development*, *140*, 943–950. doi:10.1242/dev.086363

Lombardo, M.C., Graziano, M.L., Polacco, J.C., & Lamattina, L. (2006). Nitric oxide functions as a positive regulator of root hair development. *Plant Signaling & Behavior*, *1*, 28–33. doi:10.4161/psb.1.1.2398

Navas, P., & Gomez-Diaz, P. (1995). Ascorbate free radical and its role in growth control. *Protoplasma*, *184*, 8–13. doi:10.1007/BF01276895

Normanly, J., Slovin, J.P., & Cohen, J.D. (1995). Rethinking auxin biosynthesis and metabolism. *Plant Physiology*, *107*, 323–329. doi:10.1104/pp.107.2.323

Olmos, E., Kiddle, G., Pellny, T.K., Kumar, S., & Foyer, C. H. (2006). Modulation of plant morphology, root architecture, and cell structure by low vitamin C in *Arabidopsis thaliana*. *Journal of Experimental Botany*, *57*, 1645–1655. doi:10.1093/jxb/erl010

Pagnussat, C.G., Lanteri, M.L., Lombardo, M.C., & Lamattina, L. (2004). Nitric oxide mediates the indole acetic acid induction activation of a mitogen activated protein kinase cascade involved in adventitious root development. *Plant Physiology*, *135*, 279–286. doi:10.1104/pp.103.038554

Pagnussat, G.C., Lanteri, M.L., & Lamattina, L. (2003). Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. *Plant Physiology*, *132*, 1241–1248. doi:10.1104/pp.103.022228

Radice, S., Perelman, P.E., & Caso, O.H. (1999). Propagación clonal de diversos portainjertos del género *Prunus* para la Pampa Deprimida. *Phyton – International Journal of Experimental Botany*, *64*, 149–156.

Rowland, A., Murray, A.J., & Welburn, A.R. (1985). Oxides of nitrogen and their impact upon vegetation. *Reviews on Environmental Health*, *5*, 295–342.

Saini, S., Sharma, I., Kaur, N., & Pati, P.K. (2013). Auxin: A master regulator in plant root development. *Plant Cell Reports*, *32*, 741–757. doi:10.1007/s00299-013-1430-5

Salmi, M.L., Morris, K.E., Roux, S.J., & Porterfield, D.M. (2007). Nitric oxide and cGMP signaling in calcium-dependent development of cell polarity in *Ceratopteris richardii*. *Plant Physiology*, *144*, 94–104. doi:10.1104/pp.107.096131

Sarropoulou, V., Dimassi-Theriou, K., & Therios, I. (2015). Effects of exogenous indole-3-butyric acid and myo-inositol on in vitro rooting, vegetative growth and biochemical changes in leaves and roots in the sweet cherry rootstock MxM 14 using shoot tip explants. *Theoretical and Experimental Plant Physiology*, *27*, 191–201. doi:10.1007/s40626-015-0044-4

Skůpa, P., Opatrný, Z., & Petrášek, J. (2014). Auxin biology: Applications and the mechanisms behind. In P. Nick & Z. Opatrný (Eds.), *Applied plant cell biology, plant cell monographs* (Vol. 22, pp. 69–102). Berlin, Heidelberg: Springer-Verlag. doi:10.1007/978-3-642-41787-0_3

Smirnoff, N. (2000). Ascorbic acid: Metabolism and functions of a multi-facetted molecule. *Current Opinion in Plant Biology*, *3*, 229–235. doi:10.1016/S1369-5266(00)00069-8

Strader, L.C., & Bartel, B. (2011). Transport and metabolism of the endogenous auxin precursor indole-3-butyric acid. *Molecular Plant*, *4*, 477–486. doi:10.1093/mp/ssr006

Wink, D.A., & Mitchell, J.B. (1998). Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. *Free Radical Biology and Medicine*, *25*, 434–456. doi:10.1016/S0891-5849(98)00092-6

Xu, Y., Zhu, X., Chen, Y., Gong, Y., & Liu, L. (2013). Expression profiling of genes involved in ascorbate biosynthesis and recycling during fleshy root development in radish. *Plant Physiology and Biochemistry*, *70*, 269–277. doi:10.1016/j.plaphy.2013.05.041

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