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Effect of some growth regulators on *in vitro* pollen germination in *Boerhavia diffusa* L.

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ABSTRACT

Boerhavia diffusa L. is an important medicinal plant and is being over exploited and faces the danger of becoming endangered. Studies on its reproductive biology are of utmost importance for its conservation. Pollen germination *in vitro* is used to test the viability of fresh pollen. Growth regulators are used to enhance pollen germination in both *in vitro* and *in vivo.* Present study has been undertaken to study the effect of some plant growth regulators, naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), gibberellic acid (GA3) and kinetin (Kn), myo-inositol (MO), polyvinylpyrrolidone (PVP), 2,4-Dichlorophenoxyacetic acid (2,4-D) and salicylic acid (SA) on *in vitro* pollen germination in *Boerhavia diffusa*. The results obtained have demonstrated that addition of various concentrations of PVP**,** SA, IAA and NAA to the control medium (6% sucrose) enhanced *in vitro* pollen germination. On the other hand, supplementing the control medium with different concentrations of MO and Kn and GA3 failed to enhance pollen germination. However, with respect to 2, 4-D addition of lower concentration the control medium enhanced pollen germination.

Keywords: Growth regulators**,** Myo-inositol, Polyvinylpyrrolidone, Salicylic acid.

Boerhaavia diffusa L. *nom. cons* (Nyctaginaceae) is commonly known aspunarnava, meaning that it rejuvenates or renews the body. It has several important medicinal properties (Bhowmik *et al*. 2012). It is used as anti-inflammatory, to cure amavataand rheumatoid arthritis, in several eye ailments, in heart diseases, anemia and edema. It is also used in Ayurveda to treat several disorders (Bhowmik *et al.* 2012). Due to its high medicinal value this plant is over-harvested by destructive collection techniques and further, habitat loss to crop-based agriculture, industrialization and urbanization, it is under the danger of being added to the list of endangered species. Therefore, there is an urgent need for finding out ways for both *in situ* and *exsitu* conservation. Reproductive biology plays an important role in both *in vitro* and *exsitu* conservation (Moza and Bhatnagar 2007), and pollen germination and pollen tube growth are key events in plant sexual reproduction and are prerequisites for fertilization and seed development. Pollen germination *in vitro* is a reliable method to test the viability of fresh pollen grains; it also addresses many basic questions in sexual reproduction and particularly useful in wide hybridization.

Present investigation was undertaken to study the effect of naphthalene acetic acid (NAA), indole-3-acetic acid (IAA), gibberellic acid (GA3), kinetin (Kn), MYO-inositol (MO), polyvinylpyrrolidone (PVC) and salicylic acid (SA) on *in vitro* pollen germination in *Boerhaavia diffusa.*

MATERIALS AND METHODS

Boerhavia diffusa growing in the fields of Agricultural Farm of Dayalbagh Educational Institute, Agrawas used in the present study. Pollen grains were collected from fresh opened flowers with dehisced anthers at 06:00-07:00 h.

Hanging drop culture technique of Brewbaker and Kwack (1963) as described by Shivanna and Rangaswami (1992) was used to study *in vitro* pollen germination. In our earlier study, maximum *in vitro* pollen germination was observed in 6% sucrose solution (Chaudhary *et al.* 2020) and this sucrose solution has been used as the control medium in the present study. Culture media consisted of control medium (6% sucrose) supplemented with different concentrations of 2, 4-D, NAA, IAA, Kinetin, GA3, myo-inositol; polyvinylpyrrolidone (PVP) and salicylic acid (SA). A drop of culture medium was placed on a clean cover glass. Fresh collected pollen grains were dusted over the cover glass and it was carefully inverted over the cavity of the slide. At the periphery of cavity, small amount of vaseline was placed leaving some spaces. The slides in five replicates were placed inversely over a pair of glass rods in a humid chamber where moist filter paper was placed inside the lid in the laboratory for 24 h (Shivanna and Rangaswami 1992). Pollen germination was observed using a light microspope at ×100 magnification after 24 h incubation period. For better visualization of germinating pollen grains, a drop of 0.5% acetocarmine was placed over the medium droplet and extra stain was removed with the help of dried piece of filter paper. Randomly selected visual areas, including about 100 pollen grains, were counted in five replicates.

Statistical Analysis—Data obtained on the effect of various concenrations of different substances on the percentage of *in vitro* pollen germination were statistically analyzed for significant difference between control and concentrations (Rice 2006).

RESULTS AND DISCUSSION

Effect of various supplements on *in vitro* pollen germina-tion in *B. diffusa* is described in the following paragraphs.

Myo-inositol (MO)—Myo-inositol, a carbocyclic sugar, mediates cell signal transduction in response to a variety of hormones. In humans it is naturally synthesized from glucose, while in germinating pollen MO readily gets converted in tube wall pectin. It was observed that supplementing control medium (6% sucrose) with 100 µm MO only 11% pollen germinated as compared to 43.05±7.3% observed in the control medium. Addition of higher concentrations of MO in the control medium drastically inhibited *in vitro* pollen germination (Table 1). Chen *et al.* (1977) observed the effect of 2-O, C-Methylene-myo-inositol (MMO), a Myo-inositol (MI) antagonist, inhibits *in vitro* pollen germination and tube elongation in *Lilium longiflorum* cv. Ace. Addition of 5 mM MMO in Dickinson's pentaerythritol medium partially blocked germination and pollen tube growth

Table 1— Effect of different concentrations of Myo—inositol on *in vitro* pollen germination after 24 h incubation.

Medium	Pollen germination (%)
Control (6% sucrose)	43.05±7.3
Control + Myo-inositol (mg l^{-1})	
Control + 50	7.4±1.02
Control + 100	11.0± 1.23
Control + 150	1.45± 0.89
Control + 200	1.50±0.83

Number of pollen grains = 450, ± SD= Standard deviation,

Polyvinylpyrrolidone (PVP)—It is commonly called polyvidone or povidone and is a water-soluble polymer made from the monomer *N*-vinylpyrrolidone (Haaf *et al.* 1985). PVP is used as a food additive or wetting agent. PVP owing to its polarity is used to bind polar molecules, as a blocking agent during southern blot analysis and for absorbing polyphenols during DNA purification (Sapir *et al*. 2016). Present study clearly demonstrated that addition of various concentrations of PVP to control medium (6% sucrose) enhanced pollen germination (Table 2). In the medium supplemented with 10 mg l^{-1} PVP significantly higher *in vitro* pollen germination (79.95%) was observed. However, addition of 2, 4, and 6 mgl^{-1} PVP in control medium exhibited only 20, 43 and 52% pollen germination, respectively. Babbar and Gupta (1982) demonstrated the beneficial effects of PVP in anther cultures. They observed that incorporation of 2, 4 D and 6 mg l^{-1} of polyvinylpyrrolidone (PVP) to MS medium, supplemented with 15 % CM, enhanced plantlet yield in anther cultures of *Datura metel.* Abdelwahd *et al*. (2008) pre-treated faba bean seeds (after removing seed coat) with 1000 mg l^{-1} for 1h polyvinylpyrrolidone (PVP), and cultured on tissue culture media. Treating the overnight soaked seed followed by culturing in Murashige and Skoog medium (MS medium) improved shoot regeneration. Sapir *et al*. (2016) described the properties of PVC in solvent systems.

Table 2—Effect of different concentrations of PVP on *in vitro* pollen germination after 24 h incubation.

Medium	Pollen germination (%)
Control (6% sucrose)	43.05±7.3
Control + PVP (mg l^{-1})	
Control + 2	20.19±3.2
Control + 4	43.71±6.5
Control + 6	52.2±7.8
Control + 10	*79.95±8.2

Number of pollen grains = 468, ± SD = standard deviation, *Significant at P = 0.05

Salicylic acid (SA)—Salicylic acid is a phenolic phytohormone found in plants with roles in plant growth and development (*Vlot et al. 2009)*. Percentage of pollen germination was enhanced by salicylic acid in a concentration dependent manner (Table 3). The basal medium supplemented with 1, 5, 10 and 25mgl^{-1} SA there was 20, 43, 52 and 60% pollen germination, respectively, and increase in the germination percentage was significantly higher in the media containing 10 and 100µM SA as compared to that in the basal medium alone (Table 3).

Table 3—Effect of different concentrations of salicylic acid (SA) on *in vitro* pollen germination after 24 h incubation..

Medium	Pollen germination (%)
Control (6% sucrose)	43.05±7.3
Control + SA (µM)	
Control + 1	20.19±3.2
Control + 5	43.71±6.5
Control + 10	*52.2±7.8
Control + 25 µM	*59.95±8.2

Number of pollen grains=468, ± SD = Standard deviation, *Significant at P = 0.05

Effect of SA on *in vitro* pollen germination has not been studied extensively. Guohui *et al.* (2010) studied the effects of polyamines, MGBG and salicylic acid on pollen germination and pollen tube growth of Yali pear and Xuehuali pear (*Pyrus bretschneideri* Rehd.). Rong *et al*. (2016) have recorded that salicylic acid (SA) and methylated SA (MeSA) alter the apical activation of ROP1 GTPase, a key regulator of tip growth in pollen tubes, in an opposite manner. Duoyan *et al.* (2016) reported that salicylic acid regulates pollen tip growth through an NPR3/NPR4-independent pathway. Interestingly, both MeSA methylesterase and SA methyltransferase, which catalyze the interconversion between SA and MeSA, are localized at the apical region of pollen tubes, indicative of the tip-localized production of SA and MeSA which is consistent with their effects on the apical cellular activities.

2, 4–Dichlorophenoxyacetic acid (2, 4-D)—It is usually referred by its common name 2, 4-D and is one of the oldest and most widely available systemic herbicides. In the present investigation, interestingly, 2, 4-D showed a hormesis effect on pollen germination at the lower concentration. The addition of 10µM 2,4-D induced significantly higher *in vitro* pollen germination (58.98±6.5%) as compared to the basal medium alone 48.5% (Table 3). However, with increase in the concentration of 2,4-D, there was a gradual reduction in pollen germination and only 9.48 ± 2.3 % germination was recorded at 100 µM of 2,4 D (Table 4). Similar observations have also been recorded by Kamble (2006) in *Hibiscus cannabinus.* Lower concentrations of 2, 4-D promoted pollen germination but higher concentrations inhibited both pollen germination and pollen tube growth.

Table 4—Effect of different concentrations of 2,4-D on *in vitro* pollen germination after 24 h incubation

Medium	Pollen germination (%)
Control (6% Sucrose)	43.05±7.3
Conrol+2,4-D (µM)	
Control + 10	*58.98±6.5
Control + 25	36.12±0.7
Control + 50	21.6± 1.2
Control +100	9.48±2.3

Number of pollen grains =446; ± SD=Standard deviation; *Significant at P=0.05

Naphthalene acetic acid (NAA) —It is an organic compound and the most used synthetic auxin in plant propagation (Hartmann *et al.*2011). NAA is widely used in agriculture as a rooting agent for vegetative propagation of plants from stem and leaf cuttings and is also used in plant tissue culture. The effect of this synthetic auxin on *in vitro* pollen germination is shown in Table 5. Pollen germination was enhanced in a concentration dependent manner and culture medium containing 50µM and 100µM NAA exhibited significantly higher pollen germination (52.2±7.8 and 59.95±8.2% respectively) as compared to germination in basal medium alone 43.05±7.1%.

Table 5—Effect of different concentrations of NAA on *in vitro* pollen germination after 24 h incubation

Medium	Pollen germination (%)
Control (6% sucrose)	43.05±7.3
Control +NAA (µM)	
Control +1	20.19±3.2
Control +10	43.71±6.5
Control + 50	*52.2±7.8
Control +100	*59.95±8.2

Number of pollen grains = 468, ± SD = Standard deviation; *Significant at P=0.05

Indole-3-acetic acid (IAA)—It is the most common naturally occurring important growth stimulating plant hormone. Chemically, it is a heterocyclic compound also known as plant auxin. Several studies on the effect of IAA on *in vitro* pollen germination and pollen tube growth in a wide variety of plants have been made. The effect of various concentrations of IAA on *in vitro* pollen germination is given in Table 6. The addition of IAA to the media enhanced pollen germination in a concentration dependent manner and with increase in the concentration of IAA, percentage of pollen germination also increased. In the medium containing only 0.01µ M IAA there was lowest germination (20.19±3.2%) and with 10 and 100µM in the media, the percentage of pollen germination was significantly enhanced (62.2±7.8 and 72.5±8.2% respectively) as compared to that in basal medium (6% sucrose).

Table 6.—Effect of different concentrations of IAA on *in vitro* pollen germination after 24 h incubation

Medium	Pollen germination (%)
Control (6% sucrose)	43.05±7.3
Control + IAA (µM)	
Control + 0.01	20.19±3.2
Control + 0.1	43.71±6.5
Control +10	*62.2±7.8
Control +100	*72.5±8.2

Number of pollen grains = 468, ± SD = Standard deviation; *Significant at P = 0.05

Kinetin (Kn)—It is a cytokinin, a class of plant hormone that promotes cell division Kinetin is often used in plant tissue culture for inducing formation of callus (in conjunction with auxin) and to regenerate shoots from callus (with lower auxin concentration). Effect of various concentrations of Kn supplemented to control medium (6% sucrose) failed to enhance *in vitro* pollen germination (Table 7). In the culture medium consisting of control medium (6% sucrose) supplemented with the highest amount of Kn (100 µM) there was only 25.05±2.1% germination, suggesting an inhibitory effect.

Table 7—Effect of different concentrations ofKinetin (Kn) on *in vitro* pollen germination after 24 h incubation.

Medium	Pollen germination (%)
Control (6% sucrose)	43.05±7.3
Control+ Kn (µM)	
Control + 0.01	5.30±0.1
Control + 0.1	6.34±0.4
Control + 10	8.55±1.1
Control +100	25.05±2.1

Number of pollen grains = 502, ± SD = Standard deviation,

Gibberellic acid—It is also called gibberellin A3, GA, and GA3This hormoneis found in plants and fungi (Silva *et al.* 2013). Gibberellins have a number of effects on plant development and they stimulate rapid stem and root growth, induce mitotic division in the leaves of some plants, and

increase seed germination rates (Edwards 1976). Various concentrations of GA3 supplemented to control medium (6% sucrose) failed to enhance *in vitro* pollen germination(Table 8). Maximum pollen germination (25.05±2.1%) was observed in the control medium containing highest concentration of GA3 (100µM), which was still much lower than that in the control medium (43%).

Table 8—Effect of different concentrations of GA3 on *in vitro* pollen germination after 24 h incubation

Medium	Pollen germination (%)
Control (6% sucrose)	43.05±7.3
Control + GA3 (µM)	
Control + 1	5.30±0.1
Control + 5	6.34±0.4
Control + 10	8.55±1.1
Control + 100	25.05±2.1

Number of pollen grains = 502, ± SD =standard deviation, *Significant at P = 0.05

Patel and Mankad (2014) have reviewed the types of media for *in-vitro* pollen germination. Pollen germination on artificial media is widely used as a test of viability, especially for bicellular pollen. Adequate media are now available for germination studies in many species with two-celled pollen grains. Plant growth regulators are released as secondary metabolites which contribute to the growth promoting effects and enhance early emergence of pollen tubes.

Earlier studies made by several investigators have recorded both inhibitory as well as growth promoting effects of NAA, IAA and GA3 in some plant species. Wu *et al*. (2008) have observed that IAA and GA, stimulated *in vitro* pollen tube growth, ABA inhibited pollen tube growth, and zeatin (ZT) had no significant effect on the process. The stimulating effect of exogenous IAA was particularly distinct, and led to synchronous growth of straighter and more slender pollen tubes compared with the controls. The studies made by Zechmann *et al.* (2011) have demonstrated that glutathione synthesis is essential for *in vitro* pollen germination. Abdelgadir *et al.* (2012) have found that pollen germination was significantly higher in an agar-based medium composed of sucrose, boric acid and calcium nitrate compared with the control and indole-3-acetic acid (IAA) treatments. Maita and Sotomayor (2015) found significant positive effects of brassinolide, GA3and kinetin in pollen germination of almonds.As in the case of *B. diffusa* in the present study, NAA did not improve pollen germination in grapevine cultivars (Gokbayrak and Engin 2016).On the other hand, Kovaleva *et al.* (2016) have recorded the participation of IAA and ABA in the osmoregulation of germinating *in vitro Petunia* pollen. Their results on the role of K^+ ions in the control of water-driving forces for transmembrane water transport allowed formulating the hypothesis that IAA and ABA stimulate germination of pollen grains and pollen tube growth. Radović *et al.* (2016) have studied the effects of auxin (IAA and gibberellin) GA3 on *in vitro* pollen germination and pollen tube growth in five almond cultivars. Pollen germination in the control variant (without application of hormones) ranged from 23.56% to 51.81%. Plant hormones enhanced pollen germination and the effect of these hormones was manifested on the length of pollen tubes. The pollen tube growth was enhanced by 23 to 86% in IAA and in the presence of gibberellins by 6 to 22%, depending on the cultivar. However, the cultivar Nessebar was an exception showing inhibition in pollen tube growth to gibberellin.

Gokbayraka and Engin (2016) have studied the effects of naphthalene acetic acid (NAA), gibberellic acid (GA3), and epibrassinolide (EBr) on *in vitro* pollen germination of three cultivars of *Vitis vinifera*The basic media contained 1% agar and 20% sucrose. Pollen germination of cultivar 'Amasya Beyazı' was not affected by the treatments and the germination rate varied between 6.16% (1.0 mg l^{-1} NAA) and 35.98% (50 mg l^{-1} GA3). The stimulating effect of GA3 and EBr was noticeable in cultivar 'Müşküle'. Concentration of 25 mg L^{-1} GA3 had the best effect in increasing pollen germination of cultivar 'Kozak Beyazı' as comparedto NAA, EBr and the control. These results showed that the response of the pollen grains to growth hormones is cultivar specific and substance specific. In general, NAA was the growth regulator that least enhanced the germination of grapevine pollen.

The results obtained during the course of present investigation in *B. diffusa* clearly demonstrate that addition of polyvinylpyrrolidone**,** salicylic acid, IAA and NAA in the media significantly enhanced *in vitro* pollen germination. On the other hand, basal medium supplementing with different concentrations of myo-inositol, Kn and GA3in the basal medium failed to enhance *in vitro* pollen germination. However, 2, 4-D at lower concentration enhanced pollen germination while at high concentrations an opposite effect was observed.

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REFERENCES

Abdelwahd R, Hakam N, Labhilili M and Udupa SM 2008. Use of an adsorbent and antioxidants to reduce the effects of leached phenolics in *in vitro* plantlet regeneration of faba bean. *AfricanJ. Biotech.* **7**(8) 997-1002.

Abdelgadir HA, Johnson SD and Staden JV 2012. Pollen viability, pollen germination and pollen tube growth in the biofuel seed crop *Jatropha curcas* (Euphorbiaceae). *South African J. Bot.* **79** 132-139.

Babbar SB and Gupta SC 1982. Promotory effect of Polyvinylpyrrolidone and L-Cysteine-HCl on pollen plantlet production in anther cultures of *Datura metel. Zeitschriftfür Pflanzen.* **106**(5) 459-464.

Bhowmik D, Sampath KP, Srivastava S, Shravan P, Sankar Aand Dutta D 2012. Traditional Indian Herbs: Punarnava and its Medicinal Importance. *J. Pharmacognosy and Phytochemistry*. **1**(1) 52–57.

Brewbaker JL and Kwack BH 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot.* **50** 859-865.

Chaudhary G, Shrivastav P and Dantu PK 2020. Pollen germination studies in *Boerhavia diffussa* Linn. (Nyctaginaceae) – an important medicinal plant. *Int. J. Plant Rep. Biol*. **12**(1) 80-83 DOI 10.14787/ijprb.2020 12.1.80-83

Chen M, Loewus MW and Loewus FA 1977. Effect of a myo-Inositol Antagonist, 2-O, C-Methylene-myo-Inositol, on the metabolism of myo-Inositol-2-^{3}H and D-Glucose-1-^{14}C in *Lilium longiflorum* pollen. *Plant Physiol*. **59**(4) 658–663. doi: 10.1104/pp.59.4.658

Duoyan RN, Mollet JC, Xuanming Liu and Yang Z 2016. Salicylic acid regulates pollen tip growth through an NPR3/NPR4-Independent Pathway. *Molecular Plant* **9**(11) 1478-1491.

Edwards M 1976. Dormancy in Seeds of Charlock (*Sinapis arvensis* L.). *Plant Physiol. **58**(5) 626–630.* doi*:*10.1104/ pp.58.5.626. PMC 542271. PMID 16659732.

Gokbayraka Z and Engin H 2016. Effect of plant growth regulators on *in vitro* pollen germination of grapevine cultivars. *Acta Hortic.* **1139** 405-408. doi10.17660 /ActaHortic.

Guohui QI, Ji-Zhong XU, Jian-Zhu S and Rui-hong H 2010. Effects of polyamines, MGBG and salicylic acid on pollen germination and pollen tube growth of Yali pear and Xuehuali pear (*Pyrus bretschneideri Rehd.*). *J. Agric. Univ. Hebei.* **31** 21-27.

Haaf F, Sanner A and Straub F 1985. Polymers of N-Vinylpyrrolidone: Synthesis, Characterization and uses. *Polymer J.* **17** 143-152. doi: 10.1295/polymj.17.143

Hartmann DE, Kester FT, Davies Jr and Geneve RL 2011. *Plant propagation principles and practices,* 8th edn. Prentice Hall, Englewood Cliffs.

Kamble SI 2006. Effect of herbicide 2, 4-D on pollen germination *in vitro* and pollen tube length of *Hibiscus cannabinus* Linn. *Biosci. Biotech. Res. Asia* **3** 477-480.

Kovaleva LV, Voronkov AS, Zakharova EV, Minkina YuV, Timofeeva GV and Andreev IM 2016. Exogenous IAA and ABA stimulate germination of petunia male gametophyte by activating Ca^{2+} – dependent K^{+} – channels and by modulating the activity of plasmalemma H^{+}-ATPase and actincytoskeleton. *Russ. J. Dev. Biol.* **47** 109–121.

Maita S and Sotomayor C 2015. The effect of three plant bioregulators on pollen germination, pollen tube growth and fruit set in almond [Prunus dulcis (Mill.) D.A. Webb] cvs. Non Pareil and Carmel. *Electronic J. Biotech*. **18** 381–386. doi:10.1016/j.ejbt.2015.07.004

Moza MK and Bhatnagar AK 2007. Plant reproductive biology studies crucial for conservation. *Curr. Sci* .**92**(9) 1207.

Patel RG and Mankad AU 2014. *In vitro* pollen germination- A review. *Inter. J. Sci. Res*. **3**(5) 304-307.

Radović A, Nikolić D, Milatović D, Zivković B and Stevanović N 2016. The effect of plant hormones on pollen germination and pollen tube growth of almond cultivars. *Acta Hort*. **1139** 375-379.

Rice JA 2006. *Mathematical Statistics and Data Analysis* (3rd edition). Duxbury Advanced.

Rong D, Luo N, Mollet JC, Liu X and Yang Z 2016. Salicylic acid regulates pollen tip growth through an NPR3/NPR4-independent pathway. *Mol Plant* **9** 1478–1491.

Sapir L, Stanley CB and Harries D 2016. Properties of Polyvinylpyrrolidone in a Deep Eutectic Solvent. *J. Phys. Chem. A*. **120**(19) 3253–3259. doi:10.1021/acs.jpca.5b11927

Shivanna KR and Rangaswami NS 1992. *Pollen Biology: A Laboratory Manual.* Springer, New Delhi.

Silva ALL, Rodrigues C, Costa JL, Machado MP, Penha RO, Biasi LA, Vandenberghe LPS and Soccol CR 2013. Gibberellic acid fermented extract obtained by solid-state fermentation using citric pulp by *Fusarium moniliforme:* Influence on *Lavandula angustifolia* Mill. cultivated in vitro(PDF). *Pak. J. Bot.* **45** (6) 2057–2064.

Vitagliano C and Viti R 1989. Effects of some growth substances on pollen germination and tube growth in different stone fruits. *Acta Hort.* **239** 62.

Vlot AC, Dempsey DA and Klessig DF 2009. Salicylic Acid, a multifaceted hormone to combat disease. *Ann Rev. Phytopathol.* **47** 177–206. doi*:*10.1146 / annurev.phyto.050908.135202. PMID 19400653.

Wu Juanzi, Qin Æ Yuan and Zhao Æ Jie 2008. Pollen tube growth is affected by exogenous hormones and correlated with hormone changes in styles in *Torenia fournieri* L. *Plant Growth Regul*. **55** 137-148 DOI 10.1007/s10725-008-9268-5

Zechmann B, Koffler BE and Russell SD 2011. Glutathione synthesis is essential for pollen germination *in vitro*. *BMC Plant Biol*. **11** 54.