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Review

The use of activated charcoal in grapevine tissue culture

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Summary

The microporous structure, the extremely large inner surface associated with adsorptive capacity and evidence of positive effects of activated charcoal (AC) in plant tissue culture media were published in several reviews (Pan and van Staden, 1998, Thomas 2008, Ahmadian *et al.* 2013). However a detailed overview on its application for grapevine is still lacking. In this review we present a comprehensive scale of results about the application of AC in different areas of grapevine tissue culture such as micropropagation, embryo rescue, somatic embryogenesis, gene transfer, protoplast culture, cryopreservation, viroid and virus elimination. These summarized data of positive results are aimed at stimulating a more extensive and effective use of AC as a powerful and beneficial component of grapevine tissue culture media.

K e y w o r d s : embryo culture; *in vitro* culture; micropropagation; protoplast culture; tissue culture media; virus elimination; *Vitis*.

A b b r e v i a t i o n s : AC: activated charcoal; IBA: indole-3-butyric acid; PEG: polyethylene-glycol; PGRs: plant growth regulators; SE: somatic embryo.

Introduction

Activated charcoal (AC) has unique physical and chemical properties since due to its porous structure AC has an extremely large inner surface with high adsorbing capacity. This adsorbing property makes AC beneficial in several fields of plant tissue culture as well (Thomas 2008). AC is widely used not only in plant tissue culture (Pan and van Staden, 1998, Thomas 2008), but also in media for isolation and maintenance of different bacteria (Atlas 2010), for DNA isolation from diverse soils (Devi *et al.* 2015) and RNA isolation from plants (Rowhani *et al.* 1993, Rajakani *et al.* 2013). Its advantageous effects are attributed to its specificity of adsorbing toxic substances and secondary metabolites accumulated in the medium (Dussert *et al.* 1992). AC is able to alter the pH and to provide a darkened environment in medium similar to soil conditions (Ahmadian *et al.* 2013). AC may also reduce inhibitory substances such as ethylene and phenolics by adsorption thus improving plant development (Pan and van Staden 1998). Its radical impact is a decrease in concentration of plant growth regulators (PGRs), vitamins and metal ions (Ahmadian *et al.* 2013).

The application of AC in grape tissue culture usually is not essential, but it could be vital in media for certain purposes, and also for some genotypes. These specific experiences are very important for the effective work in grapevine *in vitro* cultures. It has been observed that the addition of AC increases the survival rate of zygotic embryos obtained during embryo rescue (Gribaudo *et al.* 1993, Valdez 2005), decreases or eliminates the browning process in protoplast cultures (Reustle and Natter 1994) or in embryogenic cultures on selection medium after co-cultivation in gene transfer experiments (Kikkert *et al.* 1996, Mozsár *et al*. 1998). AC increased the efficiency of rooting and the length of the roots (Poudel *et al.* 2005, Shinde *et al.* 2010). AC promoted effectively the somatic embryo (SE) differentiation and development (Martinelli *et al.* 2001, Gambino *et al.* 2005), secondary embryo initiation (Scorza *et al.* 1995, Li *et al.* 2001), or post-culturing of cryopreserved cells (Dussert *et al.* 1991, Wang *et al.* 2005).

On the other hand AC was not essential for the enhancement of rooting frequency (Roubelakis-Angelakis and Zivanovitc 1991, Dev *et al.* 2015). Its application proved to be negative sometimes on plating efficiency in protoplast cultures or callus induction (Reustle and Natter 1994, Novák *et al.* 2011) or strongly modified the effect of PGRs during the regeneration process (Mozsár *et al.* 1998).

The positive effect of AC for grape tissue culture has also been mentioned in several book chapters (Bouquet *et al.* 2007, Martinelli and Gribaudo 2009, Carimi *et al.* 2012), but in our best knowledge a detailed review has not been published yet. Plant tissue culture is widely applied both in research and in practice, e. g. in gene transfer studies, for production of pathogen free plants and rapid propagation of new cultivars and clones. Therefore increasing the efficiency of *in vitro* cultures to which AC may signi-

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ficantly contribute, has a key importance both for scientific and practical purposes, especially for vegetatively propagated plants like grapevine. Here we would like to review the application of AC in various fields of grapevine *in vitro* cultures.

The use of AC in various fields of grapevine *in vitro* culture

Micropropagation: The amount of AC in micropropagation varies between 0.1 and 30.0 g·L^{-1} (Tab. 1). AC was successfully used in rooting media, or during the shoot multiplication and maintenance. The effect of AC on rooting frequency is genotype dependent, but the addition of low amounts of AC (100-200 mg·L^{-1}) usually increased the root length. The addition of 200 mg·L^{-1} AC was found to be beneficial for enhancing the rooting and minimizing the time to root initiation in different genotypes (Alizadeh *et al.* 2010). After 8 weeks of inoculation onto rooting medium the best root development was obtained in half strength MS medium with 2.0 mg·L^{-1} indole-3-butyric acid (IBA) and 200 mg·L^{-1} AC (Lazo-Javalera *et al.* 2016). Addition of AC was essential not only for enhancing the rooting frequency, but also for improving overall root quality, important for subsequent *ex vitro* survival (Dev *et al.* 2015).

AC alone or with IBA did not improve rooting frequency of *Vitis ficifolia* var. *ganebu* and its interspecific hybrid but significantly increased root length of the mostly single rooted plants (Poudel *et al.* 2005). Addition of AC at 3.0 % in MS medium did not increase the number of rooted explants (Roubelakis-Angelakis and Zivanovitc 1991). The use of AC slightly reduced the rooting from 92.71 % to 90.86 % and the time of root initiation increased from 14.65 d to 16.12 d, but the hormone content of the compared media was different (Itoo *et al.* 2013). When AC was applied at 3.0 g·L^{-1} only 20 % of shoots formed roots, while at 200 mg·L^{-1} AC more extensive root initiation was obtained after 6 weeks of growth (Lazo-Javalera *et al.* 2016).

MS medium supplemented with IBA (2.0 mg·L^{-1}) and AC (200 mg·L^{-1}) resulted in the best shoot multiplication results (Dev *et al.* 2015). In another experiment, addition of 200 mg·L^{-1} AC to the proliferation medium was not advantageous in case of 'SO4' rootstock (*V. berlandieri* × *V. riparia*), because some of the explants did not proliferate, although remained alive for a long period. The reduction of AC concentration to 100 mg·L^{-1} recovered shoot proliferation (Alizadeh *et al.* 2010). We found the same inhibitory effect of AC on 'Muscat Ottonel' and 'Gesztus' cultivars maintained on solid half strength MS medium containing 2.0 g·L^{-1} AC, while other cultivars ('Richter 110', 'Szirén' and 'Trilla') were successfully propagated on the same medium for several months (our unpublished observations). After 3-4 months the shoot growth of 'Szirén' and 'Trilla' plants became slower on 2.0 g·L^{-1} AC, thus the maintenance was continued on AC-free medium.

Taken together, the lower amount of AC (100-500 mg·L^{-1}) in the medium effectively helped the rooting process and 100-200 mg·L^{-1} seems to have no inhibitory effect on shoot proliferation.

Ovule and zygotic embryo culture: Successful culture and plant recovery of abortive ovules from pollinated stenospermocarpic grapes have been reported in many cultivars (Park *et al.* 1999). The technique

Table 1

Use of AC in grape micropropagation

Plant material	Results and observations	AC (g·L^{-1})	Reference
ARG 1 (Aramon × Rupestris Ganzin 1), Cabernet Franc, Cardinal, Couderc 1613 (complex interspecific hybrid), Italia, Liatiko, Loose Perlette, Mandilari, Rhazaki, Richter 99 (*V. berlandieri* × *V. rupestris*), Richter 110 (*V. berlandieri* × *V. rupestris*), Rupestris du Lot (*V. rupestris*), Ramsey (V. *champinii*), SO4 (*V. berlandieri* × *V. riparia*), Thompson Seedless	addition of AC at 3.0% in MS medium did not increase the number of one-node segments producing roots	30	Roubelakis-Angelakis and Zivanovitc 1991
Ugni Blanc	multiplication through *in vitro* microcuttings	0.5	Robert *et al.* 2001, Robert *et al.* 2002
Kadainou R-1 (*V. ficifolia* × *V. vinifera*), *Vitis ficifolia* var. *ganebu*	increased root length	0.2	Poudel *et al.* 2005
Couderc 3309 (*V. riparia* × *V. rupestris*), Dogridge (*Vitis rupestris* × *V. candicans*), SO4 (*V. berlandieri* × *V. riparia*), H-144 (*V. vinifera* × *V. labrusca*)	rooting	0.2	Alizadeh *et al.* 2010, Alizadeh *et al.* 2012
Thompson Seedless	rooting	0.2	Shinde *et al.* 2010
Pixie (dwarf)	*in vitro* propagation and maintainance	0.5	Nicholson *et al.* 2012
Perlette	root length	0.2	Itoo *et al.* 2013
BRS Clara (complex interspecific hybrid), Crimson Seedless	culture initiation with individual stem segments to obtain rooted plantlets for induction of autopolyploidy	2.5	Sinski *et al.* 2014
Hybrid 76-1 (Hur × Cardinal), Julesky Muscat, Pearl of Csaba, Pusa Navrang	shoot proliferation and rooting	0.2	Dev *et al.* 2015
Flame Seedless	rooting	0.2	Lazo-Javalera *et al.* 2016

of *in ovulo* embryo rescue consists of aseptic ovule removal and culture, embryo excision and plantlet formation. *In vitro* embryo growth and germination can be promoted by AC in media (Liu *et al.* 2003). The amount of AC in ovule and zygotic embryo culture varies between 0.02 and 3.00 g·L^{-1} (Tab. 2). AC was used successfully for direct germination of seeds, and in ovule and embryo cultures on solid, in liquid or double phase media, and less often for plantlet development and rooting.

Tsolova (1990) reported that embryos when cultured on medium containing 3.0 g·L^{-1} AC showed higher viability. In the work of Gribaudo *et al.* (1993) 2.0 g·L^{-1} AC decreased the percentage of blackened, degenerated ovules, but did not reduce callusing. It has also been observed that addition of AC increases the survival rate of developing embryos. Burger and Goussard (1996) reported that significantly more embryos were obtained with 'Muscat Seedless' and 'Festival Seedless' ovules when cultured eight weeks after bloom on medium supplemented with AC irrespective of basal medium. Depending on culture starting date after bloom, the addition of AC to the medium resulted in higher numbers of embryos. *In vitro* embryo survival and plantlet formation were higher for torpedo-shaped embryos, and it was greatly improved in 6-benzyladenine (BA)-supplemented woody plant (WP) medium containing 3.0 g·L^{-1} AC. Incorporation of AC in WP medium almost doubled embryo survival and resulted in a mean 3.5-fold increase in plantlet formation (Liu *et al.* 2003).

The role of AC was not clear, but it may be explained by the dark environment similar to the conditions under which embryos grow inside ovules before dissection (Liu *et al.* 2003). Due to the high concentration of phenolic compounds in grape ovules, the addition of AC can help in the removal of inhibitory substances and promote embryo recovery (Li *et al.* 2015). In addition, when medium containing AC was used to culture zygotic embryos, somatic embryogenesis was observed in some cultures (Liu *et al.* 2003, Bharathy and Agrawal 2008).

We may conclude that 1.0-3.0 g·L^{-1} AC effectively promotes embryo cultures and embryo formation and sometimes the whole regeneration process was executed on AC containing media (Tab. 2).

Somatic embyogenesis: AC has already been used for culturing different types of grapevine tissues (López-Pérez *et al.* 2005). The amount of AC during somatic embryogenesis changes between 0.5 and 3.0 g·L^{-1} (Tab. 3), and was effective in the steps of plant regeneration for almost 70 grape genotypes. AC was used successfully for embryo differentiation, development, germination, often for embryo proliferation and less often for callus maintenance (Tabs 3 and 4), plantlet development and rooting.

Addition of 2.5 g·L^{-1}AC during callus induction on anthers (Perl *et al*. 1995) resulted in embryogenic cultures on various cultivars ('Centennial Seedless', 'Novomuscat Seedless', 'Ruby Seedless', 'Superior Seedless'), while in other experiments 2.5 g·L^{-1} AC totally inhibited embryogenic callus induction in the case of 'Chardonnay' (Novák *et al.* 2011).

The addition of AC to the medium proved to be essential for SE differentiation and maturation in several *Vitis* cultivars (Martinelli and Gribaudo 2009). Independently of the callus induction medium used, calli cultured on

Table 2

Use of AC for grape embryo rescue

Pollinated cultivars and hybrids	Results and observations	AC (g·L^{-1})	Reference
Thompson Seedless	*in ovulo* embryo culture	0.02	Emershad and Ramming 1984
Kishmish Moldavski	limiting the necrosis of cultivated embryos	3.0	Tsolova 1990
Argentina, Carina, Centennial Seedless, Imperatrice, Flame Seedless, Perlon, Perlette, Ruby Seedless	*in ovulo* embryo culture	2.0	Gribaudo *et al.* 1993
Muscat of Alexandria	ovule culture in liquid medium	1.0	Okamoto *et al.* 1993.
Festival Seedless, Flame Seedless, Muscat Seedless	ovule culture	2.0	Burger and Goussard 1996
Dawn Seedless, Ruby Seedless, Superior Seedless	direct germination of seeds, or embryo excision, or seed coat was ruptured	2.7	Valdez and Ulanovsky 1997
triploid interspecific hybrids	*in ovulo* embryo culture	1.0	Yamashita *et al.* 1998
triploid interspecific hybrids	immature seed culture (in the first month)	2.0	Park *et al.* 1999
Marroo Seedless (complex interspecific hybrid), Merbein Seedless, Sunmuscat	embryo survival, recovery and plant formation	3.0	Liu *et al.* 2003
Flame Seedless	ovule and embryo culture, embryo germination	3.0	Bharathy *et al.* 2005
seedless grapevine cultivars	immature embryo rescue	2.7	Valdez 2005
Thompson Seedless	ovule and zygotic embryo culture, repetitive somatic embryogenesis on zygotic embryos	3.0	Bharathy and Agrawal 2008
Blush Seedless, Delight, Emerald Seedless, Flame Seedless, Monukka, Ruby Seedless, Thompson Seedless	*in ovulo* culture (double phase medium) embryo culture and plant regeneration	3.0 2.0	Tian *et al.* 2008
Centennial Seedless, Thompson Seedless	ovule culture in liquid medium	3.0	Tang *et al.* 2009
Crimson Seedless, Flame Seedless, Ruby Seedless, Thompson Seedless	embryo formation on double phase medium, embryo germination and plantlet development	3.0	Li *et al.* 2014
Blush Seedless, hybrids	embryo formation embryo culture and germination rooting medium	3.0 1.0 1.5	Li *et al.* 2015

Table 3

Use of AC in somatic embryogenesis for different grapes

Plant material	Results and observations	AC ($g \cdot L^{-1}$)	Reference
Chancellor (complex interspecific hybrid)	SE conversion to plantlets	3.0	Hébert-Soulé *et al.* 1995
Centennial Seedless, Novomuscat Seedless, Ruby Seedless, Superior Seedless	embryogenic callus production, culturing of embryogenic clusters and callus, embryo development, dedifferentiation	2.5	Perl *et al.* 1995
Thompson Seedless	subculturing embryogenic callus and cells	2.0	Compton and Gray 1996
Brachetto a Grappolo Lungo, Chardonnay	embryo differentiation	2.5	Martinelli *et al.* 2001
Fredonia (*V. labrusca*), Niagara (complex interspecific hybrid)	embryo development and maturation	2.5	Motoike *et al.* 2001
Pinot Meunier	formation, proliferation and germination of embryos	2.5	Franks *et al.* 2002
Chardonnay, Thompson Seedless	SE production	0.5	Jayasankar *et al.* 2003
Barbera, Brachetto a Grappolo Lungo, Chardonnay, Kober 5 BB (*V. berlandieri ×V. riparia*), Moscato Bianco, Müller-Thurgau, Richter 110 (*V. berlandieri ×V. rupestris*), Riesling, Rupestris du Lot (*V. rupestris*)	embryo differentiation	2.5	Martinelli *et al.* 2003
Barbera, Chardonnay, Richter 110 (*V. berlandieri* × *V. rupestris*)	formation and proliferation of embryos	2.5	Gribaudo *et al.* 2004
1103 Paulsen (*V. berlandieri ×V. rupestris*), Crimson Seedless, Dominga, Don Mariano, Italia, Red Globe, Sugraone	embryo development and germination	2.5	López-Pérez *et al.* 2005
Cabernet Sauvignon, Chardonnay, Fercal (complex interspecific hybrid), Gewürztraminer, Grenache, Merlot, Sauvignon Blanc	embryo differentiation	2.5	Maillot *et al.* 2006, Maillot *et al.* 2009 Maillot *et al.* 2016
Cabernet Sauvignon, Richter 110 (*V. berlandieri* × *V. rupestris*), Kober 5 BB (*V. berlandieri ×V. riparia*), Kober 125 AA (*V. berlandieri ×V. riparia*), Tempranillo	plant regeneration from elongated embryos	no data	Ben-Amar *et al.* 2007
Brachetto a Grappolo Lungo, Chardonnay, Grignolino, Müller Thurgau, Richter 110 (*V. berlandieri* × *V. rupestris*)	embryo proliferation	2.5	Gambino *et al.* 2007
Autumn Royal Seedless	somatic embryogenesis and plant regeneration	3.0	Jittayasothorn *et al.* 2007
Touriga Nacional	embryo differentiation medium	3.0	Pinto-Sintra 2007
Carménère	embryo development and germination	2.5	Cadavid-Labrada *et al.* 2008
Autumn Seedless, Barbera, Blanc du Bois (complex interspecific hybrid), Cabernet Franc, Cabernet Sauvignon, Chardonnay, Conquistador (complex interspecific hybrid), Dolcetto, Emerald Seedless, Freedom (complex interspecific hybrid), Harmony (complex interspecific hybrid), Merlot, Pinotage, Pinot Gris, Pinot Noir, Ramsey (V. *champinii*), Richter 110 (*V. berlandieri* × *V. rupestris*), Riparia Gloire (*V. riparia*), Rupestris du Lot (*V. rupestris*), Sauvignon Blanc, Semillon, Seyval Blanc (complex interspecific hybrid), Shiraz, Stover (complex interspecific hybrid), Superior Seedless, Tampa (complex interspecific hybrid), Thompson Seedless, White Riesling, Zinfandel	development of proembryonic masses (PEM) and SEs. Further PEM and SE proliferation occurred on X6 medium. Maintenance of embryogenic cultures.	0.5	Dhekney *et al.* 2009b
Lumassina, Provinè	embryo differentiation	2.5	Gambino *et al.* 2010
Albariño, Brancellao, Mencía, Merenzao, Torrontés, Treixadura	callus proliferation, embryo differentiation and germination, secondary embryogenesis	2.5	Prado *et al.* 2010a, Prado *et al.* 2010b, Acanda *et al.* 2013, Acanda *et al.* 2015
Alachua (*M. rotundifolia*), Carlos (*Muscadinia* interspecific crossing), Darlene (*M. rotundifolia*), Delicious (*M. rotundifolia*), Supreme (*Muscadinia* interspecific crossing)	maintenance of embryogenic cultures, development of SEs, direct secondary embryogenesis	0.5	Dhekney *et al.* 2011
BRS Clara (complex interspecific hybrid), Crimson Seedless	embryo proliferation for induction of autopolyploidy	2.5	Sinski *et al.* 2014
Manicure Finger	embryo development and plant regeneration	0.5	Xu *et al.* 2014
Pinot Noir	embryo germination, shoot and root development	3.0	Larrouy *et al.* 2016
Richter 110 (*V. berlandieri* × *V. rupestris*)	embryo differentiation in liquid medium embryo development and germination on solid medium	1.0 2.0	Forgács *et al.* 2017
Thompson Seedless	induction of somatic embryos	1.0	Ji *et al.* 2017

medium with AC showed differentiated embryos after 20-25 d while embryogenesis was delayed or absent in half strength MS without AC (López-Pérez *et al.* 2005). The authors found that addition of AC (2.5 g·L^{-1}) to the medium used for callus culture was an essential prerequisite for forming SEs in the case of 'Crimson Seedless', 'Italia' and 'Don Mariano' cultivars; it greatly increased the frequency of embryogenic callus formation in 'Sugraone' from 5.8 % to 99.5 %. Moreover, the number of embryos per callus increased as well. AC also had a significantly positive effect on induction and embryo development of *Vitis labrusca* SEs (Motoike *et al.* 2001). Proembryonic masses regenerated SEs when they were transferred to medium containing AC, and a significantly positive interaction was observed between PEG (polyethylene-glycol) and AC. Ethylene is antagonist of auxin, and because of that AC adsorbing ethylene is able to promote embryo development (Jona *et al.* 2002). Development and proliferation of SEs on X6 medium containing 0.5 g·L^{-1} AC occurs by direct secondary embryogenesis, with new embryos emerging from epidermal or sub-epidermal cells (Jayasankar *et al.* 2003, Dhekney *et al.* 2011).

Addition of IBA and 0.5 mg·L^{-1} AC to the medium accelerated the growth of plants, with 72 % of them having at least six true leaves within the culture period. The positive effect of AC may be due to its ability to adsorb inhibitors such as aromatic compounds produced by plant tissues or residual growth regulators which are inhibitory to plant growth or development (Xu *et al*. 2014)

Considering the above data we can conclude that 0.5-3.0 g·L^{-1} AC can effectively help the embryo development and often the embryo proliferation process. Sometimes the whole regeneration process is carried out on AC containing media with similar concentrations (Tab. 3).

Gene transfer into grapevine: Gene transfer into grapevine is mostly initiated on embryogenic cultures, thus AC is frequently involved in the different steps of this process. AC was added from 0.5 to 3.0 g·L^{-1} to the culture media in case of 44 tested cultivars (Tab. 4). AC containing media were successfully applied for induction of embryogenic cultures (Scorza *et al.* 1996) or indirect organogenesis (Guan *et al.* 2013), maintenance of embryogenic calli (Perl *et al.* 1996, Hanson *et al.* 1999), embryo development and proliferation (Li *et al.* 2001, Dhekney *et al.* 2009a), co-cultivation (Scorza *et al.* 1995, Le Henanff *et al.* 2011), selection (Torregrosa *et al.* 2002), embryo germination (Vidal *et al.* 2003, Kikkert *et al.* 2005), plant regeneration (de la Torre *et al.* 2012) and rooting (Dai *et al.* 2015).

Efforts to maintain embryogenic callus in GS1CA (Franks *et al.* 1998) medium containing 2.5 g·L^{-1} AC were unsuccessful, but pre-embryogenic cultures were maintained for a year on PT medium containing 0.5 g·L^{-1}. For both 'Chardonnay' and 'Thompson Seedless', the number of calli that grew on selection medium and the number of plants obtained was higher on PT medium containing 0.5 g·L^{-1} compared to NB medium without AC, while 'Rupestris du Lot' callus grew better on NB medium lacking AC (Agüero *et al.* 2006). Compared to other media, callus cultured on GS1CA (2.5 g·L^{-1} AC) became heterogeneous in few weeks. Embryogenic callus subcultured onto this medium 3-4 weeks before co-culture increased transformation competence (Torregrosa *et al.* 2002).

Bouquet *et al.* (2007) proposed the use of 2.5 g·L^{-1} AC for induction of SEs and for co-cultivation on solid medium, while other authors omit AC from the co-cultivation medium (Jiao *et al.* 2017). In the experiments of Mozsár *et al*. (1998) AC proved to be effective to reduce browning of calli, but it also strongly modified the impact of the PGRs added to the medium probably by adsorbing them. Therefore, in subsequent experiments they used insoluble polyvinylpyrrolidone (PVP) to reduce necrotic responses of explants. Addition of AC (2.0 g·L^{-1}) to the culture medium for embryo germination and plantlet development improved the yield of normal plantlets (Geier *et al.* 2008).

In summary, 2.0-3.0 g·L^{-1} AC was used for stimulation of somatic embryogenesis on leaves and zygotic embryos, 0.5-3.0 g·L^{-1} AC for embryo development and proliferation of somatic embryos, 2.5-3.0 g·L^{-1} AC for co-cultivation, 0.5-3.0 g·L^{-1} AC for selection after co-cultivation, 1.5-3.0 g·L^{-1} AC for germination, 0.5-3.0 g·L^{-1} AC for shoot development and 1.0-3.0 g·L^{-1} AC for rooting (Tab. 4).

Grapevine protoplast culture: In grapevine protoplast cultures 1.5-10.0 g·L^{-1} AC was used to promote cell division, microcallus and embryo development (Tab. 5). Ui *et al.* (1990) reported an improvement in the rate of cell division of grapevine protoplasts by adding 2.0 g·L^{-1} AC to the culture media. In the work of Reustle and Natter (1994) the applied AC concentrations were high enough to avoid browning process of the media. They observed negative effects of AC on plating efficiency. Development of microcalli occurred only with 5.0 and 10.0 g·L^{-1} AC, but at very low frequencies. Zhu *et al*. (1997) published that for the continuous growth of the colonies without browning, it was essential to add 3.0 g·L^{-1} AC in the liquid reservoir medium from the beginning of the culture, both plating efficiency and number of embryos obtained were ten times higher than those obtained without AC. The highest plating efficiency and colony formation were obtained when AC was added to the culture medium from the beginning of protoplast culture.

Cryopreservation of grapevine cells: The use of AC varied from 1.0 to 3.0 g·L^{-1} in cryopreservation experiments with grapevine cells (Tab. 6). Solid post-culture medium containing 2.5 g·L^{-1} AC was found to promote viability of cryopreserved cells (Wang *et al.* 2002). Dussert *et al.* (1992) observed that in presence of AC the regrowth of grape cells decreased. Nevertheless AC containing media were used for 13 different cultivars after thawing for regrowth, post-culturing and embryo proliferation.

In vitro virus elimination: Somatic embryogenesis is an effective tool for viroid (Gambino *et al.* 2011) and virus elimination (Gambino *et al.* 2006, Gribaudo *et al.* 2006) of grapevine. The lack of vascular connection between grapevine SEs and the parent tissue was considered to be the reason for the sanitation (Peiró *et al.* 2015). For the elimination process it is not necessary

Table 4

Use of AC in grape gene transfer experiments

Plant material	Results and observations	AC ($g.L^{-1}$)	Reference
Three *V. vinifera* hybrids	stimulation somatic embryogenesis from zygotic embryos and proliferation of SEs, co-cultivation, selection, germination	3.0	Scorza *et al.* 1995
Chancellor (complex interspecific hybrid)	reduced cell browning on selection medium, embryo development, rooting of embryos, shoot development	3.0	Kikkert *et al.* 1996
Red Globe	long term maintenance of embryogenic calli and SEs	2.5	Perl *et al.* 1996
Thompson Seedless	SE production on leaves and culturing embryo germination and development into rooted plants	2.0 3.0	Scorza *et al.* 1996
Thompson Seedless	formation and proliferation of SEs, co-cultivation, germination	2.5	Franks *et al.* 1998
Georgikon 28 (complex interspecific hybrid)	reduced browning of embryogenic material after co-cultivation	3.0	Mozsár *et al.* 1998
Emperor	maintaining embryogenic callus, selection medium	0.5	Hanson *et al.* 1999
Couderc 3309 (complex interspecific hybrid), 101-14 MGT (complex inter-specific hybrid), Richter 110 (*V. berlandieri* × *V. rupestris*), Riparia Gloire (*V. riparia*), Teleki 5C (*V. berlandieri* × *V. riparia*)	rooting	3.0	Xue *et al.* 1999
41 B MGT (*V. vinifera* × *V. berlandieri*)	mature plant development	0.5	Radian-Sade *et al.* 2000
Superior Seedless	plant regenaration from putative transformed calli	2.0	Gollop *et al.* 2001
Cabernet Sauvignon, Chardonnay, Chenin Blanc, Muscat Gordo Blanco, Pinot Noir, Riesling, Sauvignon Blanc, Semillon, Shiraz	GS1CA medium 1 month prior to transformation, co-cultivation, callus proliferation during selection, germination	2.5	Iocco *et al.* 2001
Merlot, Seyval Blanc (complex interspecific hybrid), Shiraz, Thompson Seedless	secondary embryo initiation, embryo induction, SE culture maintenance	0.5	Li *et al.* 2001, Li *et al.* 2004, Li *et al.* 2006, Li *et al.* 2008
Chardonnay, Danuta, Portan, Shiraz	proliferation of embryogenic callus, co-cultivation, subculturing, selection	2.5	Torregrosa *et al.* 2002
Chardonnay	embryo induction and germination	3.0	Vidal *et al.* 2003, Kikkert *et al.* 2005
Blaufränkisch, Lumassina, Nebbiolo	embryo differentiation	2.5	Gambino *et al.* 2005
Chardonnay, Rupestris du Lot (*V. rupestris*), Thompson Seedless	subculturing pre-embryogenic calli, subculturing on selection medium	0.5	Agüero *et al.* 2006
Alachua (*M. rotundifolia*), Carlos (*Muscadinia* interspecific crossing)	cultures were maintained by careful transfer of proliferating proembryonal masses to fresh X6 medium	0.5	Dhekney *et al.* 2008
Thompson Seedless	SE germination, rooting	1.5	Fan *et al.* 2008
Richter 110 (*V. berlandieri* × *V. rupestris*)	germination and plantlet development	2.0	Geier *et al.* 2008
Cabernet Franc, Cabernet Sauvignon, Chardonnay, Conquistador (complex interspecific hybrid), Freedom (complex interspecific hybrid), Harmony (complex interspecific hybrid), Merlot, Orange Muscat, Pinot Noir, Ramsey (V. *champinii*), Richter 110 (*V. berlandieri* × *V. rupestris*), Riparia Gloire (*V. riparia*), Rupestris du Lot (*V. rupestris*), Sauvignon Blanc, Seyval Blanc (complex interspecific hybrid), Shiraz, Superior Seedless, Thompson Seedless, Zinfandel	SE development and proliferation	0.5	Dhekney *et al.* 2009a
Thompson Seedless	induction and maintenance embryogenic cultures using liquid media	0.5	Tapia *et al.* 2009
Albariño, Grenache, Tempranillo, Thompson Seedless	embryo induction	3.0	Vidal *et al.* 2009
Couderc 3309 (complex interspecific hybrid), Richter 110 (*V. berlandieri* × *V. rupestris*), Teleki 5C (*V. berlandieri* × *V. riparia*)	shoot and root formation of elongated hypocotyls of SEs, plant regeneration	3.0	Krastanova *et al.* 2010
Chardonnay	co-cultivation, three weeks culturing to remove contaminating *Agrobacterium*, SE production	2.5	Le Henanff *et al.* 2011
Prime Seedless	subculturing and maintenance of embryogenic callus	2.5	Zhao *et al.* 2011a
Chardonnay	maintenance of embryogenic and non-embryogenic callus	2.5	Zhao *et al.* 2011b
Albariño	embryo development and plant regeneration	3.0	de la Torre *et al.* 2012
Chardonnay	embryo development	2.5	Jelly *et al.* 2012

Tab. 4, continued

Plant material	Results and observations	AC ($g.L^{-1}$)	Reference
V. pseudoreticulata Baihe-35-1 x *V. vinifera* Carignane	micro shoot tips, stems with single buds for callus formation (indirect organogenesis) and adventitious buds induction, elimination of *Agrobacterium* and shoot development	1.0	Guan *et al.* 2013
Thompson Seedless	embryo differentiation and secondary embryogenesis	1.0	Zhou *et al.* 2014
Sugraone	embryo differentiation	2.5	Dabauza *et al.* 2015
Chardonnay	rooting	1.0	Dai *et al.* 2015
Sauvignon Blanc	initiation of shoot tip cultures to obtain *in vitro* plantlets for transient transformation of leaf tissue	3.0	Lizamore and Winefield 2015
Thompson Seedless	embryo development	0.5	Rubio *et al.* 2015
Thompson Seedless	plant development from germinated embryos	1.0	Cheng *et al.* 2016
Thompson Seedless	subculturing on selection medium	0.5	Jiao *et al.* 2017

Table 5

Use of AC in grape protoplast culture

Plant material	Results and observations	AC ($g \cdot L^{-1}$)	Reference
Kosyu	cell division and microcallus development	2.0	Ui *et al.* 1990
Vidal Blanc (complex interspecific hybrid)	prevention of browning and inhibition of development of protoplasts (bilayer system)	1.0-10.0	Reustle and Natter 1994
Koshusanjaku	cell division, growth of the colonies without browning, embryo development	3.0	Zhu *et al.* 1997

Table 6

Use of AC in cryopreservation of grape cells

Plant material	Results and observations	AC ($g \cdot L^{-1}$)	Reference
41 B MGT (*V. vinifera* × *V. berlandieri*)	18 days post-culturing after rapid thawing	1.0	Dussert *et al.* 1991
41 B MGT (*V. vinifera* × *V. berlandieri*), Chardonnay	the presence of AC reduced the regrowth of cryopreserved cells.	1.0	Dussert *et al.* 1992
41 B MGT (*V. vinifera* × *V. berlandieri*), Chardonnay, Gammy, Red Globe, Richter 110 (*V. berlandieri* × *V. rupestris*), Sugarone	post-culturing of cryopreserved cells regeneration from individual embryos at torpedo stage	2.5 2.5	Wang *et al.* 2004, Wang *et al.* 2002
Red Globe	post-culturing of cryopreserved cells rooting of genetic modified 4 mm long embryos originated from cryopeserved cell lines of Red Globe	2.5 2.5 2.5	Wang *et al.* 2005
Albariño, Tempranillo	7 days post-culturing after thawing embryo development	2.5 3.0	Gonzalez-Benito *et al.* 2009
Chardonnay, Dauphine, Merlot, Red Globe, Roobernet, Thompson Seedless	embryo proliferation from the cryopreserved beads	2.5	Vasanth and Vivier 2011
Richter 110 (*V. berlandieri* × *V. rupestris*), Riesling, Tempranillo	regrowth of embryogenic masses	2.5	Ben-Amar *et al.* 2013

to apply long term maintenance of the embryogenic cultures, and the use of 2.0-2.5 $g \cdot L^{-1}$ AC can greatly promote the successful embryo differentiation. Up to now 11 virus and viroid free cultivars were obtained on AC containing media in case of five viruses and two viroids (Tab. 7). No negative effect of AC was observed on the effectiveness of virus elimination. In our laboratory use of 2.0 $g \cdot L^{-1}$ AC in culture medium showed positive effect in case of weakly developing plantlets originating from meristem cultures of several cultivars (our unpublished observation).

Concluding remarks

The total number of cultivars and other genotypes for which AC containing media were used in the cited research articles for different purposes is more than hundred. This fact shows the importance of AC in grape tissue culture. AC has a serious positive impact on zygotic and SE development and secondary embryogenesis, and this ability was utilized in zygotic embryo cultures, gene transfer or virus elimination. The protective effect of AC can help in protoplast cultures, cryopreservation and during the selection in gene transfer.

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Table 7

Use of AC during viroid and virus elimination from grapes

Cultivars	eliminated viroids and viruses	Results and observations	AC ($g \cdot L^{-1}$)	Reference
Bosco, Grignolino, Müller-Thurgau	GRSPaV, GLRaV-1, GVA, GLRaV-3	maintenance of embryogenic cultures	2.5	Gambino *et al.* 2006
Albarola, Bosco, Brachetto, Grignolino, Müller Thurgau, Rossese, Vermentino	GRSPaV	embryo differentiation	2.5	Gribaudo *et al.* 2006
Cari, Proviné, Roussan	GFLV	embryo differentiation	2.5	Gambino *et al.* 2009
Cari, Proviné, Roussan, Nebbiolo	GYSVd-1, HSVd	embryo differentiation	2.5	Gambino *et al.* 2011
Grumet Negre	GLRaV-1, GLRaV-3	embryogenic callus induction on transversally cutted seeds, embryo development	2.0	Peiró *et al.* 2015

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