sciendo

DOI: 10.2478/ahr-2018-0010

Acta Horticulturae et Regiotecturae 2
Nitra, Slovaca Universitas Agriculturae Nitriae, 2018, pp. 42–47

EFFECTS OF GIBBERELLIC ACID AND LOW TEMPERATURE ON GERMINATION OF SOME *PRUNUS* SPECIES EMBRYOS (WITHOUT COTYLEDONS) UNDER LABORATORY CONDITIONS

Mahmoud. A. GHAYYAD*

Damascus University, Syria

Seeds of *Prunus* species do not germinate as a result of different mechanisms of dormancy such as physiological, physical and/or chemical ones. This study was carried out in order to determine the effects of three concentrations of Gibberellic acid (GA_3) 1, 3, and 5 mg.L^{-1} and low temperature at 5 °C on germination and on the length of isolated embryos from cotyledons of almond, apricot, plum, peach, mahaleb and sweet cherry on top of filter paper under laboratory conditions. The highest germination percentage (96.67%) was at 1 mg.L^{-1} GA_3 without cold treatment or 3 mg.L^{-1} GA_3 + cold treatment in almond, the highest length of embryos (15.47 mm) was also in almond at 1 mg.L^{-1} GA_3 without cold treatment. Embryos of mahaleb and sweet cherry germinated at low germination percentages of 31.16%, 33.33% respectively at 3 mg.L^{-1} GA_3 + cold treatment. It was concluded that embryos of almond, apricot, plum and peach were germinated successfully on top of filter paper under laboratory conditions and GA_3 increased significantly the germination percentages of the isolated embryos either after cold treatment or without compared with controls. A strong positive correlation was found between seed germination without testa, embryos germination and final embryos length simultaneously.

Keywords: *Prunus*, isolated embryos, GA_3, cold treatment, filter paper, laboratory conditions

Seeds of *Prunus* species do not germinate immediately when planted, either in the field or in the laboratory, as a result of different mechanisms of dormancy such as physiological (embryo), physical (endocarp), chemical (inhibitors) ones or the combinations of mentioned mechanisms; dormancy in these species may last for several months.

The germination inhibitors exist at different concentrations in various parts of seed, including pericarp, seedcoat, cotyledons, and embryo. The proportion of inhibitors could be decreased by removing either one or several parts of the seeds and thus germination percentage could be increased (San and Yildirim, 2009). On the other hand, cold stratification of seeds for several months could overcome dormancy. Also, some growth regulators such as GA_3 were used to induce the germination of the seeds of some species, for example *P. mahaleb* (Ghayyad et al., 2010; Gercekcioğlu and Cekic, 1999; Pipinis et al., 2012).

Overcoming seed dormancy *in vitro* germination was also successfully used in strawberry (Miller et al., 1992), citrus fruits (Hassanein and Azooz, 2003), walnut (Kaur et al., 2006), and almond (San and Yildirim, 2009). Germination of immature embryos has been successfully accomplished in some stone fruits including cherry (Hormaza, 1999), apricot (Ning et al., 2007), peach, almond, and peach×almond hybrids (Ledbetter et al., 1998).

A few studies have been carried out about *in vitro* germination of embryos isolated from cotyledons (Arbeloa et al., 2009; San and Yildirim, 2009), but all of the studies are based on MS medium and combinations of benzylaminopurine (BAP) and gibberellic acid (GA_3) and /or Indol butyric acid (IBA). San et al. (2014) reported that for successful *in vitro* embryo germination, the MS medium should be fortified with 0.5 mg·L^{-1} BAP + 3.0 mg·L^{-1} GA_3 in apricot, peach, and wild cherry. Payghamzadeh and Kazemitabar (2010a) reported that the best performing medium for immature embryos germination walnut was DKW basal medium supplemented with 1 mg L^{-1} alone and 1.5 mg L^{-1} BAP in conjunction with 0.01, 0.05 and 0.1 mg L^{-1} IBA (germination ratios vary between 49.32% and 67.76%). Kaur et al. (2006) reported that the best performing medium was MS with 0.5 mg.L^{-1} kinetin, 0.5 mg.L^{-1} BAP and 2 mg.L^{-1} GA_3 yielding 66.6% germination in Netar Akhrot cultivar of walnut after 12 days of culturing and the percent germination of immature embryos was higher when BAP and IBA were simultaneously applied as compared to those when applied separately (Kaur et al., 2006).

In addition, Kaur et al. (2006) used cold treatment in *in vitro* germination of immature walnut embryos, and the per cent germination of excised embryos was higher when GA_3 and cold treatments were simultaneously applied as compared to those when applied separately.

The object of this study is to determine the effects of Gibberellic acid (GA_3) and low temperature on germination of isolated embryos from cotyledons of almond, apricot, plum, peach, mahaleb and sweet cherry on top of paper under laboratory conditions without the need for nutritious medium and the easy and rapid assessment of embryos growth under simple conditions.

Contact address: Mahmoud. A. Ghayyad, Damascus University, Faculty of Agriculture, Natural Resources and Ecology Department, Syria, e-mail: ghayyad.m@gmail.com

Material and methods

This study was carried out in 2017 during Summer and Autumn in Damascus Countryside – Beit Tima Seed Scientific Research Laboratory.

Seeds of six *Prunus* species were collected: almond (*Prunus amygdalus* L.), apricot (*Prunus armeniaca* L.), plum (*Prunus domestica* L.), peach (*Prunus persica* L.), Mahaleb (*Prunus mahaleb* L.), and sweet cherry (*Prunus avium* L.). Fruits were collected in the same year of the study, then the seeds were isolated from mature fruits and dried at laboratory temperature (20–25 °C) for a month before they were used in the tests.

At first, hard endocarps were removed using a small hammer, then seeds were soaked in sterilized water for 48 hours for easy removal of testa and embryos, water being replaced every 12 hours.

After the testa (seedcoat) was removed, seeds with and without testa were incubated on top of filter paper under laboratory conditions in petri dishes without any pre-treatments as controls to compare with other treatments.

The embryos were carefully excised from the cotyledons under sterile conditions and were cultured on top of filter paper under laboratory conditions (temperature 20–25 °C, 16 hours a day and 8 hours a night) in petri dishes and submitted to the following treatments for three (3) weeks. Each treatment consisted of three (3) replications and each one contained tow (2) petri dishes with 10 embryos in each petri dish.

Gibberellic acid (GA_3)

Embryos were moistened with three (3) different concentrations (1,3, and 5 $mg.L^{-1}$) of GA_3 solutions during the three weeks test (when needed).

Cold treatment (low temperature)

Embryos were kept in the refrigerator for three (3) weeks at 5 °C and moistened with distilled water when needed, after that embryos were tested under laboratory conditions.

GA_3 + cold treatment

Embryos were moistened with the three (3) concentrations (1,3, and 5 $mg.L^{-1}$) of GA_3 solutions, then kept in the refrigerator for 3 weeks at 5 °C and moistened with GA_3 when needed, then tested in laboratory conditions.

At the end of the tests, the results of the germinated embryos were recorded in table (1) as percentages. Also, the final length of the germinated embryos (root and shoots) was measured in order to examine how different species of embryo growth are affected by the different treatments. The results were recorded in table (2).

Statistical analysis

Embryo germination percentages were transformed using ArcSin (Square root (X)). Since transformed data must meet the assumption of normality, data normality was determined by Kolmogorov-Smirnov test of variances, factorial ANOVA was performed to test the main effects and interactions between the studied factors (species 6 levels × GA_3 concentrations 4 levels × cold treatment/without cold treatment 2 levels × 3 replications). For comparing the germination of the isolated embryos with seed germination,

Table 1 Effects of GA_3 and cold treartments on embryos with and without cotyledons germination on top of filter paper under laboratory conditions

Seed germination percentage with cotyledons (%)								
Treatments		**species**						**average**
		almond	**apricot**	**plum**	**peach**	**mahaleb**	**cherry**	
Seed with testa		0	0	0	0	0	0	0
Seed without testa		81.67	60	70	66.67	35	28.33	56.94
Embryo germination percentage without cotyledons (%)								
Treatments		**species**						**average**
GA_3 ($mg.L^{-1}$)	**cold treatment 5 °C**	**almond**	**apricot**	**plum**	**peach**	**mahaleb**	**cherry**	
0	0 week	76.66bL	48.33bM	13.33cN	16.67bN	15.00bN	10.00bN	**30.00**
1	0 week	96.67aL	91.67aL	71.16aM	68.83aM	25.00aN	30.00aN	**63.88**
3	0 week	95.00aL	90.00aL	61.67aM	65.00aM	20.00aN	31.67aN	**60.55**
5	0 week	91.67aL	81.16aLM	73.33aLM	66.67aLM	21.67aN	20.00aN	**59.08**
0	3 week	40.00cL	40.00dL	36.67bL	35.00bL	13.33bM	11.67bM	**29.44**
1	3 week	83.33aL	80.00aL	80.00aL	76.67aL	31.16aM	33.33aM	**64.08**
3	3 week	96.67aL	51.67bcdN	80.00aL	70.00aM	15.00bO	11.67bO	**54.17**
5	3 week	90.00aL	60.00cbM	73.33aLM	71.67aLM	13.33bN	10.00bN	**53.05**
Average		**83.52**	**66.98**	**62.16**	**59.68**	**21.05**	**20.74**	

Means with the same letters of a, b, c, d in the same column are not significantly different at $\alpha = 0.05$. Means with the same letters of L, M, N, O in the same row are not significantly different at $\alpha = 0.05$

factorial ANOVA (species 6 levels × treatments 9 levels × 3 replications) was also performed. The Tukey HSD test at (α = 0.05) was used as a multiple comparison procedure after ANOVA analysis. Data analysis was performed by using Microsoft Excel 2007 and Statistical.8 StatSoft, Inc Program. The results were presented after back-transform data by using (Sin (*x*))2.

Results and discussion

Table 1 shows the germination percentages of seeds with cotyledons (without testa) and isolated embryos germinated on different concentrations of GA_3 and cold treatment under the laboratory conditions on top of filter paper in almond, apricot, plum, peach, mahaleb and sweet cherry.

Seeds with testa did not germinate on filter paper under laboratory conditions in all studied species. The highest germination percentage of the seeds without testa was recorded for almond, then for plum, peach, apricot, mahaleb and sweet cherry simultaneously, but according to the Tukey test at (α = 0.05) there were no significant differences between almond and plum, apricot and peach, and mahaleb and cherry.

Factorial ANOVA results showed that the final germination of the isolated embryos was significantly affected by the species ($F(5, 96) = 542.72$, $p < 0.001$), by the GA_3 concentrations ($F(3, 96) = 266.56$, $p < 0.001$), by the interaction of species and GA_3 ($F(15, 96) = 16.166$, $p < 0.001$), by the type of cold treatment ($F(1, 96) = 11.888$, $p < 0.001$), by the interaction of species and the type of cold treatment ($F(5, 96) = 30.238$, $p < 0.001$) and by the interaction of all of these factors ($F(15, 96) = 6.9483$, $p < 0.001$).

Results of the Tukey test (α = 0.05) showed that germination percentage of almond was significantly the highest followed by apricot, plum and peach, and mahaleb and cherry. Germination percentage at 1 $mg.L^{-1}$ of GA_3 was significantly the best. For the concentrations 3 and 5 $mg.L^{-1}$ there were no significant differences, control was the lowest. Germination percentages of the incubated embryos without cold treatment were significantly higher than after cold treatment.

There were no significant effects of 1, 3 or 5 $mg.L^{-1}$ concentrations of GA_3 for almond, plum and peach, meanwhile the concentration of 1 $mg.L^{-1}$ was significantly higher than the others on apricot, mahaleb and cherry.

Germination percentages of almond and apricot embryos without cold treatment were significantly higher than those after cold treatment and in reverse for plum and peach, whereas there were no significant effects on mahaleb and cherry.

The highest germination percentage (96.67%) was at 1 $mg.L^{-1}$ GA_3 without cold treatment and 3 $mg.L^{-1}$ GA_3 + cold treatment in almond. However, the differences between the germination percentages obtained from cold treatment and GA_3 were not statistically significant. The highest germination percentage (91.67%) was 1 $mg.L^{-1}$ GA_3 + cold treatment in apricot, in plum 1 or 3 $mg.L^{-1}$ GA_3 + cold

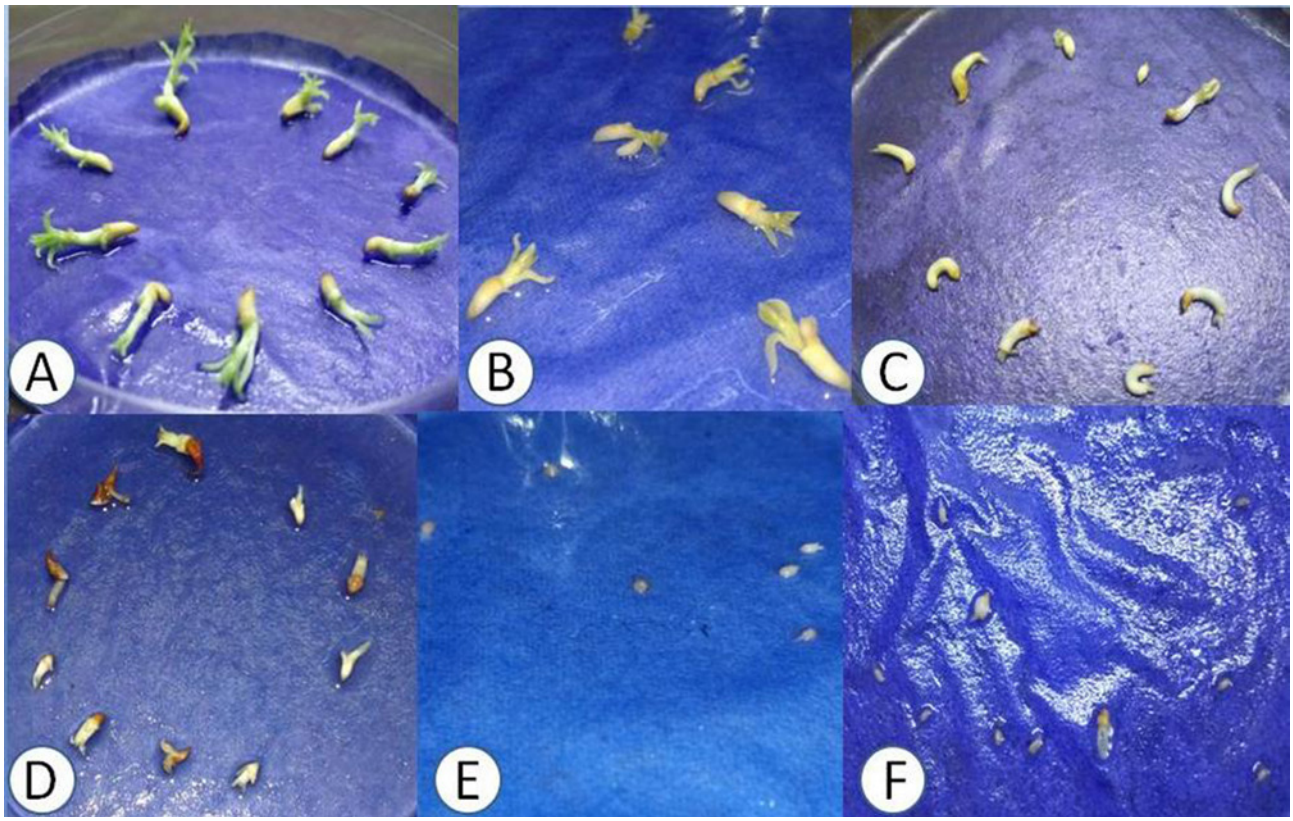

Figure 1 Embryos germination of some *Prunus* species without cotyledons on top of filter paper (FP) under laboratory conditions (A) almond embryos on FP + GA_3 1 $mg.L^{-1}$ without cold treatment. (B) apricot embryos on FP + GA_3 1 $mg.L^{-1}$ without cold treatment, (C) plum embryos on FP + GA_3 3 $mg.L^{-1}$ + cold treatment, (D) peach embryos on FP + GA_3 1 $mg.L^{-1}$ + cold treatment, (E) mahaleb embryos on FP + GA_3 1 $mg.L^{-1}$ + cold treatment, (F) sweet cherry embryos on FP + GA_3 1 $mg.L^{-1}$ + cold treatment

treatment (80%), meanwhile 1 mg.L^{-1} GA_3 + cold treatment in peach, mahaleb and cherry (76.67%, 31.16%, and 33.33% respectively), it was noted that only the root germinated in plum.

The results obtained in this study showed that seeds with testa did not germinate. Removing the testa increased the final germination percentage, this agrees with the fact that in some species, dormancy is overcomed by removing from seed coats that are likely to contain substances that inhibit embryo growth including *Malus*, *Prunus*, and *Pyrus* (Johnson and Chirco, 2003), while San et al. (2014) reported that seeds with cotyledons and/or testa of apricot, peach, and wild cherry did not germinate on the MS medium without cold treatment or stratification.

Isolated embryos of almond, apricot, plum and peach germinated on top of filter paper successfully in spite of the facts that tests were carried out under laboratory conditions (Figure 1 shows the isolated embryos from cotyledons germination of the studied species). Isolated embryos of mahaleb and sweet cherry did not germinate very satisfactorily. Likewise, seeds without testa also germinated at low germination ratios, that may be related to combined dormancy (Embryo not fully developed when seed shed with physiological germination block, Geneve, 1999). Roots of plum germinated, but shoots did not either after cold treatment or without, this may indicate to the Epicotyl dormancy in plum since the same was noted in seeds without testa test.

In all species, gibberellic acid (GA_3) significantly increased the germination percentages of the isolated embryos either after cold treatment or without comparing with controls; these results agree with Hartmann et al. (1997) in the rule of gibberellin in overcoming physiological dormancy in seeds with dormant embryos, but there was no significant effect of the addition of gibberellic acid on embryos germination; Payghamzadeh and Kazemitabar (2010a) reported that in the free PGR (plant growth regulators) medium embryos of walnut germinated, but did not induce any embryo development.

The interaction of species and cold treatment from statistical standpoint showed that germination of plum (only root) and peach isolated embryos was effected positively by the cold treatment, which means roots germinated after cold treatment, shoot remained dormant, and according to Geneve (1999) this may also indicate the Epicotyl and radicle dormancy. Meanwhile, embryos of almond and apricot were germinated better without cold treatment, there was no effect of cold treatment on mahaleb and sweet cherry embryos, the changes in the last two species were only turgidity of the root, that may relate to embryo dormancy which did not overcome completely. In all species and in general, there was no significant effect of GA_3 + cold treatment on germination embryos compared with only GA_3; this means that the main effect on germination was for GA_3, and for plum, peach, mahaleb and sweet cherry there was maximum embryo germination percentages of 80%, 76%, 67%, 31.16% and 33.33% respectively.

Embryo length (shoots and roots)

Table 2 shows the mean length of the germinated embryos for almond, apricot, plum, peach, mahaleb and sweet cherry on filter paper under laboratory conditions at the end of the tests.

Factorial ANOVA results showed that there were significant differences betweeen the length of the germinated embryos of the studied species ($F(5, 96) = 1{,}711.4$, $p < 0.001$), GA_3 concentrations ($F(3, 96) = 137.01$, $p < 0.001$) The type of cold treatment $F(15, 96) = 17.873$, $p < 0.001$), the interaction of species and the type of cold treatment ($F(5, 96) = 90.297$, $p < 0.001$), and the interaction of all of these factors ($F(15, 96) = 22.348$, $p < 0.001$).

Results of the Tukey test ($\alpha = 0.05$) showed that the highest length of germinated embryos was significant in almonds, followed by apricot and peach, plum, cherry and then mahaleb.

The highest length of the germinated embryos was shown at 1 or 3 mg.L^{-1} of GA_3 concentration, whereas the control reached the lowest length. The length of the incubated embryos without cold treatment was significantly higher compared to the cold treatment, and the length of almond, apricot, peach and cherry embryos without cold treatment was significantly higher than after cold treatment,

Table 2 The mean final length of the germinated embryos (root and shoots mm) after 3 weeks incubation

Treatments		Species						average
GA_3 (mg.L^{-1})	cold treatment 5 °C	almond	apricot	plum	peach	mahaleb	cherry	
0	0 week	11.50bL	7.14bM	6.00bM	6.17bM	1.67abN	2.67bN	**6.19**
1	0 week	15.47aL	8.50aM	6.17bN	8.33aM	3.00aO	3.56aO	**7.51**
3	0 week	14.77aL	9.10aM	50.80bN	5.58aM	2.89aP	3.89aO	**7.51**
5	0 week	12.56bL	6.92bM	7.11abM	7.61aM	2.83aN	3.5aN	**6.75**
0	3 week	7.33dL	7.25bL	4.00cM	3.42cM	2.00nN	2.00aN	**4.33**
1	3 week	9.75bcL	5.87bcN	8.13aM	6.67bN	2.56aO	2.33aO	**5.88**
3	3 week	10.70bL	4.80cO	8.25aM	6.73bN	2.33aP	3.00aP	**5.97**
5	3 week	11.11bL	5.28cM	6.19bM	6.28bM	2.67aN	2.67bN	**5.70**
Average		**11.65**	**6.86**	**6.46**	**6.72**	**2.50**	**2.95**	

Means with the same letters of a, b, c, d in the same column are not significantly different at $\alpha = 0.05$; means with the same letters of L, M, N, O, P in the same row are not significantly different at $\alpha = 0.05$

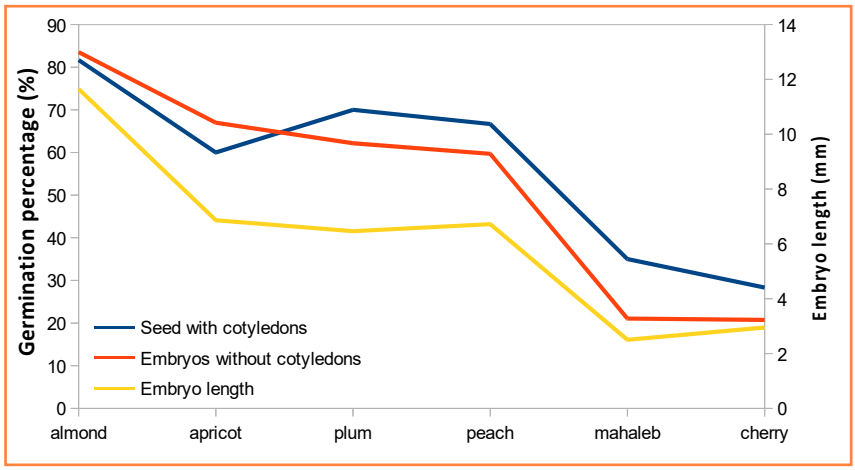

Figure 2 The mean seed germination percentages of the species with cotyledons, the mean isolated embryos germination percentages without cotyledons and the mean final embryos length

whereas there were nt significant differences in plum and mahaleb.

The highest length was recorded in almond at 1 or 3 mg.L^{-1} GA_3 without cold treatment (15.47 mm, 14.77 mm), the same for apricot, peach, mahaleb and cherry the highest length was at 3 mg.L^{-1} without cold treatment. However, there were no significant differences between 1, 3, and 5 mg.L^{-1} without cold treatment for the three previous species, for plum the highest length was at 3 or 1 mg.L^{-1} + cold treatment (8.25 mm, 8.13 mm).

Gibberellin acid (GA_3) without cold treatment had a positive effect on the development of the embryos, except for plum embryos (only root) which had the best length after cold treatment with presence of GA_3. In general, low temperatures seem to be favourable to the germination and embryo growth of plum because of the epicotyl dormancy. Payghamzadeh and Kazemitabar (2010b) reported that high frequency of plantlet of pecan embryos was reported in modified DKW basal medium supplemented with 1 mg L^{-1} BAP, 0.05 mg L^{-1} IBA and 2 mg L^{-1} GA_3 and dark culture condition.

In other study, Payghamzadeh and Kazemitabar (2010a) found out that the longest main shoot length of immature embryos of walnut was achieved in DKW medium supplemented with 1 and 1.5 mg.L^{-1} BAP.

Scaltsoyiannes et al. (1997) reported the effects of cytokinin (BA) and auxin (IBA) on shoot development.

A strong positive correlation was found between seed germination without testa (with cotyledons *x*) and embryos germination percentages of the species (*y*) ($R = 0.97$), the simple linear correlation equation was ($y = 1.19x - 15.53$). On the other hand, a positive correlation was found between embryos germination (*x*) (means of the species) and the final length of the germinated embryos (*y*) ($R = 0.95$, $y = 0.12x - 0.2$). (Figure 2 shows the relationship between seed germination with cotyledons, without cotyledons and embryos length). Subsequently, a positive correlation was found between seed germination without testa (with cotyledons *x*) and the final length of the germinated embryos (*y*) ($R = 0.92$, $y = 0.15x - 2.13$).

In this study, the strong positive correlation was found between seed germination percentage without testa (with cotyledons) and isolated embryos germination percentage, and the final length of germinated embryos; the more germination without testa, the more germination embryos without cotyledons, and the more length of embryos. These relationships may lead to the conclusion that inhibitors do not necessarily present in the cotyledons since the seeds were not stored and the inhibitors did not move from testa to the cotyledons or embryos according to the theory of the inhibitors movement during storage; in this case, the closest probability is that inhibitors are present in the embryos. Moreover, it was concluded that culturing isolated embryos from cotyledons depend on the range of the mother seed dormancy and viability.

These relationships give a clear idea about the nature of the dormancy and the approximate prediction of the embryos growth.

Conclusions

In conclusion, isolated embryos from cotyledons of almond, apricot, peach and plum (only root) were germinated successfully on top of filter paper under laboratory conditions without the need for nutritious medium.

In all species, gibberellic acid (GA_3) significantly increased the germination percentages of the isolated embryos either after cold treatment or without comparing with controls.

A strong positive correlation was found between seed germination without testa (with cotyledons), embryos germination and the final embryos length at the same time.

Acknowledgement

We gratefully thank the staff of the Seed Scientific Research Laboratory in Damascus countryside for their cooperation. This study was accomplished in spite of the hard conditions in the country.

References

ARBELOA, A. – DAORDEN, M.E. – GARCIA, E. – ANDREU, P. – MARIN, J.A. 2009. *In vito* culture of Myroplan (*Prunus cerasifera* Ehrh) embryos. In HortScience, 2009, no. 44, pp. 1672–1674.

GERCEKCIOGLU, R. – CEKIC, C. 1999. The effects of some treatments on germination of mahaleb (*Prunus mahaleb* L.) seeds. In Turkish J Agric Forestry, 1999, no. 23, pp. 145–150.

GENEVE, R.L. 1999. Seed dormancy in commercial vegetable and flower species. In Proc. Intl. Plant Prop. Soc., 1999, no. 49, pp. 248–254.

GHAYYAD, M. – KURBYSA, M. – NAPOLSY, G. 2010. Effect of endocarp removal, gibberelline, stratification and sulfuric acid on germination of Mahaleb (*Prunus mahaleb* L.) seeds. In American-Eurasian Journal of Agricultural and Environmental Science, 2010, no. 9, pp. 163–168 https://www.idosi.org/aejaes/jaes9(2)/10.pdf

HARTMANN, H.T. – KESTER, D.E. – DAVIES, F. JR. – GENEVE, R.L.1997. Plant Propagation Principles and Practices. 6th ed., New Jersey : Prentice Hall, 1997.

HASSANEIN, A.M. – AZOOZ, M.M. 2003. Propagation of Citrus reticulata via *in vitro* seed germination and shoot cuttings. In Biol. Plant., 2003, no. 47, pp. 173–177.

HORMAZA, J.I. 1999. Early selection in cherry combining RAPDs with embryo culture. In Sci. Hort., 1999, no. 79, pp. 121–126.

JOHNSON, G. – CHIRCO, E. 2003. Excised embryo tests of peach, apple and pear. Proceedings of the ISTA Forest Tree and Shrub Seed Committee Workshop Prague – Průhonice, Czech Republic, October 20–22. Forestry and Game Management Research Institute Jiloviště-Strnady, CR and Forestry Commission Research Agency, UK. http://www.vulhm.cz/sites/File/vydavatelska_cinnost/sborniky_a_dalsi_publikace/sbornik_ista.pdf

KAUR, R. – SHARMA, N. – KUMAR, K. – SHARMA, D.R. – SHARMA, S.D. 2006. *In vitro* germination of walnut (*Juglans regia* L.) embryos. In Scientia Horticulturae, 2006, no. 109, pp. 385–388. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.845.1786&rep=rep1&type=pdf

LEDBETTER, C.A. – PALMQUIST, D.A. – PETERSON, S.J. 1998. Germination and net *in vitro* growth of peach, almond, and peach-almond hybrid embryos in response to mannitol inclusion in the nutrient medium. In Euphytica, 1998, no. 103, pp. 243–250.

MILLER, A.R. – SCHEEREUS, J.C. – ERB, P.S. – CHANDLER, C.K. 1992. Enhanced strawberry seed germination through *in vitro* culture of cut achenes. In J. Amer. Soc. Hort. Sci., 1992, no. 117, pp. 313–316.

NING, G.G. – BAI, S.P. – BAO, M.Z. – LIU, L. 2007. Factors affecting plantlet regeneration from *in vitro* cultured immature embryos and cotyledons of *Prunus mume* 'Xue mei'. In *In Vitro* Cell. Dev. Biol. Plant, 2007, no. 43, pp. 225–230.

PAYGHAMZADEH, K. – KAZEMITABAR, S.K. 2010a. The effects of BAP, IBA and genotypes on *in vitro* germination of immature walnut embryos. In International Journal of Plant Production, vol. 4, 2010, no. 4, pp. 309–322. http://ijpp.gau.ac.ir/article_714_fe51cf9369d57b06b13921ab106e59b0.pdf

PAYGHAMZADEH, K. – KAZEMITABAR, S.K. 2010b. *In vitro* germination of Pecan (*Carya illinoinensis*) embryo. In Biharean Biologist, 2010, no. 4, pp. 37–43. http://biozoojournals.ro/bihbiol/cont/v4n1/bb.041106.Kazemitabar.pdf

PIPINIS, E. – MILIOS, E. – MAVROKORDOPOULOU, O. – GKANATSIOU, C. – ASLANIDOU, M. – SMIRIS, P. 2012. Effect of pretreatments on seed germination of *Prunus mahaleb* L. In Not. Bot. Horti Agrobo., vol. 40, 2012, no. 2, pp. 183–189. http://www.notulaebotanicae.ro/index.php/nbha/article/viewFile/7887/7022

SAN, B. – YILDIRIM, A.N. 2009. Seed and *in vitro* embryo germination in aged almond. In Seed Sci. and Technol., 2009, no. 37, pp. 365–371. https://doi.org/10.15258/sst.2009.37.2.10

SAN, P. – YILDIRIM, A.N. – YILDIRIM , F. 2014. An *In Vitro* Germination Technique for Some Stone Fruit Species: the Embryo Isolated from Cotyledons Successfully Germinated without Cold Pre-treatment of Seeds. In Hortscience, vol. 49, 2014, no. 3, pp. 294–296. http://hortsci.ashspublications.org/content/49/3/294.full

SCALTSOYIANNES, A. – TSOULPHA, P. – PANETSOS, K.P. – MOULALIS, D. 1997. Effect of genotype on micropropagation of walnut trees (*Juglans regia* L.). In Sil. Gen., 1997, no. 46, pp. 326–332.