OPTIMIZING MICROPROPAGATION OF APPLE (*MALUS* × *DOMESTICA* BORKH) AND *IN VITRO* ROOT INDUCTION BY *PIRIFORMOSPORA INDICA*

SAMIRA MASOUDI^{1,2}, MARYAM JAFARKHANI KERMANI^{1*}, ALI SOLEIMANI²,
HASSAN HAJNAJARI³, AMIN ALIDADI¹, ZAHRA SAADAT HOSSEINI¹

¹Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

²University of Zanjan, Zanjan, Iran

³Temperate and Cold Fruits Research Institute (TCFRI), Horticultural Science Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

MASOUDI, S. – KERMANI, M.J. – SOLEIMANI, A. – HAJNAJARI, H. – ALIDADI, A. – HOSSEINI, Z.S.: Optimizing micropropagation of apple (*Malus* × *domestica* Borkh) and *in vitro* root induction by *Piriformospora indica*. Agriculture (Poľnohospodárstvo), vol. 66, no. 4, pp. 137–147.

The inoculation of plant species with mycorrhiza fungus *Piriformospora indica* results in enhancement of growth, increase in yield, and induction of resistance to biotic and abiotic diseases through improvement of the root system. The aim of the present study was to optimize *in vitro* propagation protocol for three indigenous apples (*Malus* × *domestica*) cultivars ('Golbahar', 'Sharbati', 'Soltani Shabestari') and one commercial cultivar ('Golden Delicious'). Furthermore, the efficiency of *P. indica* at rooting stage was investigated on three cultivars ('Sharbati', 'Soltani Shabestari', 'Golden Delicious'). Establishment and proliferation stages were optimized by collecting explants at different seasons and comparing different culture media respectively. Rooting optimization included six treatments containing different concentrations of auxins in the presence or absence of *P. indica*. Results showed that at the establishment stage, a maximum percent of survival was observed in explants collected in spring. At the proliferation stage, different media had a divergent effects on distinct cultivars. Although all cultivars reacted favourable to micropropagation in MS (Murashige & Skoog 1962) basal medium, the presence or absence of cytokinin 2ip (N⁶-(2-Isopentenyl) adenine) in the culture media showed significant and incremental improvements in growth indices. In all cultivars highest rooting percent, root length, root thickness, and the number of roots/explant was observed in MS media containing auxins for three weeks followed by a treatment of MS medium containing *P. indica* for another three weeks. Plantlets treated with *P. indica*, grow stronger and healthier at the acclimation stage compared to the ones that excluded *P. indica*.

Key words: apple cultivars, culture media, establishment, proliferation

Different species or shrubs of the genus *Malus* are either found in the wild or cultivated in different regions of Iran. Among these species, *Malus domestica* Borkh has numerous modified breeds and it is cultivated as the fruit “apple” in horticulture (Mo-

zaffarian 1996). The cultivation area of apple in Iran is third after grape and pistachio, with 9.1% of the total area under cultivation of orchard products. The area under cultivation is 218,000 hectares with an average yield of 16 tons per hectare. This average

Samira Masoudi, Maryam Jafarkhani Kermani (*Corresponding author), Amin Alidadi, Zahra Saadat Hosseini, Department of Cell and Tissue Culture, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran. E-mail: maryam.j.kermani@gmail.com
Samira Masoudi, Ali Soleimani, Department of Horticulture, Faculty of Agriculture, University of Zanjan, Zanjan, Iran
Hassan Hajnajari, Temperate and Cold Fruits Research Institute (TCFRI), Horticultural Science Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

yield is too far from the leading countries with an average of 86 tons per hectare in Switzerland (FAO 2016). Part of the gap between Iran and developed countries in terms of yield tonnage per hectare is due to the lack of farmers' access to healthy seedlings, which has been resulted from the traditional propagation of pathogen-infected plants. Thus, providing large numbers of healthy high yielding apple seedlings and introducing them to the horticulture industry is quite an important issue.

In vitro micropropagation of plants is the only method to produce large numbers of pathogen-free plant material in a relatively short period of time. There are many reports on successful methods for apple micropropagation (Batelja Lodeta *et al.* 2019; Bhattacharya & Singh 2018; Kaur *et al.* 2018; Geng *et al.* 2015; Jafarkhani Kermani *et al.* 2009). Since different factors such as genotype and conditions before and during the regeneration process amongst plant growth regulators (PGRs), and culture media influence stages of *in vitro* propagation of plants, therefore, optimizing different stages of micropropagation are essential in a successful protocol.

Piriformospora indica is a mycorrhizal endosymbiont of orchid and ericoid roots, which has been reported to promote the growth of several plant species, increase yield and product quality, and induce resistance to biotic and abiotic stresses (Franken 2012). *P. indica* promoted the generation and growth of *in vivo* adventitious roots in pelargonium and poinsettia (Druege *et al.* 2007). Hua *et al.* (2017) stated that an important function of *P. indica* is auxin induction and production of its mediators. Dong *et al.* (2013) reported that this fungus plays an important role in enhancing the expression of genes that are effective in phytohormones in barley. Moreover, the effect of *P. indica* on gene expression of Chinese cabbage and *Cymbidium aloifolium* has been reported by Hua *et al.* 2017 and Shah *et al.* 2019 respectively. Liu *et al.* (2019) compared the interaction of *P. indica* with the roots of seven host plants and showed that root colonization strategies and changes in two phytohormones levels are highly host-specific.

Since the effect of the symbiont fungi *P. indica* on the rooting stage of micropropagation of apple has not been reported, the present investigation

was designed to study the effect of symbiont fungi *P. indica* on the rooting stage of two indigenous apple cultivars ('Sharbati', 'Soltani Shabestari') and a commercial cultivar ('Golden Delicious'). Moreover, establishment and shoot proliferation stages were optimized for the mentioned cultivars and another apple cultivar ('Golbahar').

MATERIAL AND METHODS

Plant material and general procedures

The plant materials were supplied by Temperate Fruits Research Center, Horticultural Science Research Institute of Iran. Stem cutting (15 cm) of three indigenous cultivars including 'Golbahar', 'Soltani Shabestari', 'Sharbati' along with a commercial cultivar, 'Golden Delicious', were collected during two growing seasons (spring and autumn). The stems were cut into 1.5 to 2 cm segments containing at least one lateral bud. They were disinfected with sodium hypochlorite (2.5%) for 12–14 min, followed by three washes in sterile distilled water.

In all experiments, media contained 30 g/L sucrose, 7 g/L agar, 100 mg/L PG (Phloroglucinol), and pH was adjusted to 5.8. The media were autoclaved at 121°C for 15 min. All the cultures were placed in growth chambers with a light intensity of 500–3,000 lux, photoperiod of 16/8 h light/dark, the relative humidity of 45%, and constant temperature of $23 \pm 1^\circ\text{C}$.

Optimizing the establishment stage

At the establishment stage, the four mentioned cultivars and two different seasons (spring and autumn) were compared. In each season 60 explants were collected twice (every time 30 explants). Explants containing at least one lateral bud were surface-sterilized, placed on MS (Murashige & Skoog 1962) basal medium, and incubated in the growth chambers. The survival (explants which grew healthy), elimination (explants that died off), and contamination (explants that were infected by bacteria or fungi) percent were recorded after one month. Moreover, the survival rate was recorded after three months too and the value change between the two records was calculated using the following formula

in the Excel program.

$$\text{Percentage change} = (A_2 - A_1) / A_1$$

* A_1 and A_2 are the percent of survival after 1 moth and 3 months respectively.

Optimizing the shoot proliferation stage

At proliferation stage three different media including; MS (Murashige & Skoog 1962), VS (van der Salm *et al.* 1994) nutrient medium, WPM (Woody Plant Medium, Lloyd & McCown in 1981) and two different concentrations (0 and 5 μmol) of 2iP (N^6 -(2-Isopentenyl) adenine) were compared. The media contained the following growth regulators; 5 μmol GA_3 (Gibberellic acid), 0.1 μmol IBA (Indole-3-butyric acid), 2 μmol BAP (6-Benzylaminopurine); added or not added 2iP. In each experiment 20 replicates (five jars with four explants) were used. Growth indices measured after six weeks included; number of axillary shoots, shoot length [cm], the number of leaves, fresh and dry weight. The fresh weight of the whole shoot was measured and then they were dried in an oven (70°C) for 72 hours before measuring dry weight.

*Investigating the effect of *P. indica* at rooting stage*

The shoots were transferred to elongation medium (MS + 5 μmol GA_3 + 0.2 μmol IBA) for four weeks prior to initiation of rooting. The rooting experiment was carried out on three cultivars ('Soltani Shabestari', 'Sharbati', 'Golden Delicious') with 15 replicates in each treatment. Each explant consisted of a 4–5 cm shoot with three leaves. The treatments were abbreviated R1 to R6 (Table 1). Some treatments contained low concentrations of auxins (low auxin medium: LAM) which included 0.5 μmol

NAA (1-Naphthalene acetic acid) and 0.5 μmol IBA (Indole-3-butyric acid), whereas other treatments contained relatively high concentrations of auxins (high auxin medium: HAM) which had 2 μmol NAA and 2 μmol IBA. In the treatments that *P. indica* (the mycorrhizal *Piriformospora indica*) was added as a rooting stimulant, 3 g of mycelia was added to every liter of culture media. *P. indica* was supplied by Agricultural Biotechnology research Institute of Iran (ABRII). The mycelia growth was achieved by placing them on 8 mm Petri dishes containing Potato Dextrose Agar in incubators at 25°C for 8 days. Rooting parameters including rooting percent, root length [cm], root thickness [cm], number of roots per explant were measured after 6 weeks.

Statistical analysis

Data were analysed in factorial based on a completely randomized design (CRD). A two-factor analysis of the genotype $\times$ medium variant was carried out for proliferation and rooting experiments. Data were analysed using the statistical program SPSS. Statistically significant averages were compared using Duncan's Multiple Range tests. Graphs were plotted with the Excel program. Differences were regarded as significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Comparing the effect of season on the establishment stage

The results of this study showed that both the genetic source and sampling time of explants affected their survival rate as well as contamination caused by bacteria and fungi at the establishment stage (Ta-

T a b l e 1

Abbreviation for different treatments at rooting stage

Treatment	Treatment Description
R1	MS basal medium (six weeks)
R2	LAM (six weeks)
R3	HAM (six weeks)
R4	MS basal medium (three weeks) + MS basal medium with <i>P. indica</i> (three weeks)
R5	LAM (three weeks) + MS basal medium with <i>P. indica</i> (three weeks)
R6	HAM (three weeks) + MS basal medium with <i>P. indica</i> (three weeks)

Note: MS – Murashige & Skoog 1962; LAM – low auxin medium; HAM – high auxin medium.

ble 2). In all cultivars, the percent of contamination was higher in autumn than in spring season, however, the number of eliminated explants due to lack of ability to grow was higher in the explants collected in spring compared to the ones collected in autumn (Figure 1). In all cultivars, the highest percent of survival was observed in explants collected in spring. Amongst the cultivars, maximum percent of survival (73.32%) was recorded in 'Golbahar'. Figure 1 illustrates that the survival rate declined after three months in all the cultivars and the decline was steeper in explants collected in autumn compared to the ones collected in spring. The highest percentage change (–48%) was observed in 'Golden

Delicious' from the samples collected in autumn. Hohtola (1988) reported that survival and percent of contaminants in the explants prepared in different seasons were affected by seasonal changes. The report showed that in pine trees contamination was considerably high in winter. Our results also suggested that the contamination rate was significantly affected by season and it was higher in explants collected in autumn than those collected in the spring. Shakyia *et al.* (2013) stated that many factors, such as the effect of soil parameters, environmental factors, phenotype and genotype of the host plant, seasons and geographic environments were involved in bacterial and fungal contamination and hence the

Figure 1. The change in survival percent after one month and three months at the establishment stage in explants of different apple cultivars collected in two seasons (The negative percentage change indicates a decrease in survival percent in month three compared to the first month).

T a b l e 2

Effect of different seasons on contamination and survival at establishment stage of apple micropropagation

Cultivar	Seasons	Contamination [%]	Elimination [%]	Survival [%] after one month	Survival [%] after three months
'Golbahar'	Spring	10.00	16.68	73.32	71.60
	Autumn	23.34	15.00	61.66	60.00
'Sharbati'	Spring	20.00	15.00	65.00	61.66
	Autumn	28.33	8.37	62.30	43.50
'Soltani Shabestari'	Spring	6.67	30.00	63.33	55.00
	Autumn	21.67	13.33	65.00	35.00
'Golden Delicious'	Spring	8.33	25.00	66.70	45.00
	Autumn	30.00	10.00	60.00	58.33

survival percent at the establishment stage of micro-propagation.

Investigating the interactive effect of culture media and cultivar type at the proliferation stage

The results of the study indicated that different culture media including Murashige & Skoog 1962 (MS); van der Salm *et al.* 1994 (VS); Woody Plant Medium (WPM) with or without 2ip (N⁶-(2-Isopentenyl) adenine) affected the growth indices signifi-

cantly in different cultivars (Table 3). The maximum number of axillary shoots (3.2) was observed in 'Golbahar' cultured on MS medium without 2ip. Whereas, the highest shoot length (5.9 cm) and the maximum number of leaves (13.9) were detected in 'Golden Delicious' cultivated on MS medium containing 2ip. The maximum amount of fresh weight (1,200 mg) was recorded in 'Golden Delicious' and 'Golbahar' in WPM and MS media without 2ip re-

T a b l e 3

Effect of different culture media on indices of explant proliferation in apple cultivars

Cultivar	Medium	No. of Axillary shoots	Mean shoot length [cm]	Mean no. of leaves	Fresh weight [mg]	Dry weight [mg]
'Golbahar'	MS	3.2 ^a	4.0 ^c	9.1 ^{def}	1,200 ^a	0.14 ^{cd}
	VS	1.1 ^{ghi}	2.4 ⁱ	5.8 ^{gh}	300 ^{gh}	0.08 ^{fgh}
	WPM	1.3 ^{fgh}	2.8 ^{fg}	6.5 ^{efgh}	200 ^h	0.06 ^{ijkl}
	MS+2ip	1.0 ^{hij}	2.9 ^{gh}	11.5 ^{ab}	400 ^{fgh}	0.10 ^{ef}
	VS+2ip	2.0 ^d	3.0 ^{efg}	9.9 ^{def}	200 ^{gh}	0.06 ^{hijk}
	WPM+2iP	2.3 ^c	2.7 ^{ghi}	7.3 ^{efg}	500 ^{cde}	0.09 ^{efg}
'Sharbati'	MS	1.5 ^{ef}	3.8 ^c	7.9 ^{def}	400 ^{fgh}	0.12 ^{de}
	VS	1.3 ^{fgh}	2.4 ^{hi}	4.5 ^h	240 ^{gh}	0.05 ^{kl}
	WPM	0.3 ^k	2.9 ^{fg}	6.3 ^{fgh}	200 ^h	0.04 ^{kl}
	MS+2ip	0.9 ^{ij}	2.9 ^{fg}	6.9 ^{efgh}	700 ^{bc}	0.23 ^b
	VS+2ip	0.9 ^{ij}	3.0 ^{efg}	6.8 ^{efgh}	400 ^{efgh}	0.06 ^{ijkl}
	WPM+2iP	1.4 ^{ef}	3.2 ^{ef}	6.3 ^{fgh}	300 ^{fgh}	0.09 ^{efg}
'Soltani Shabestari'	MS	1.0 ^{hij}	3.2 ^{ef}	10.4 ^{abcd}	500 ^{cde}	0.021 ^b
	VS	1.0 ^{hij}	2.5 ^{hi}	5.6 ^{gh}	400 ^{efgh}	0.07 ^{gi}
	WPM	1.2 ^{fgh}	2.7 ^{ghi}	11.2 ^{ab}	300 ^{fgh}	0.06 ^{ijkl}
	MS+2ip	1.2 ^{fgh}	3.2 ^{ef}	10.9 ^{abc}	1,100 ^a	0.11 ^{ef}
	VS+2ip	1.1 ^{ghi}	2.9 ^{fg}	8.4 ^{def}	400 ^{efgh}	0.06 ^{h-k}
	WPM+2ip	0.8 ⁱ	2.9 ^{fg}	7.5 ^{defg}	500 ^{efg}	0.08 ^{e-h}
'Golden Delicious'	MS	1.6 ^c	3.3 ^{de}	7.5 ^{defg}	500 ^{efg}	0.08 ^{efghi}
	VS	2.3 ^c	3.7 ^{cd}	7.0 ^{efg}	600 ^{bcd}	0.11 ^{efg}
	WPM	2.2 ^{cd}	3.9 ^c	10.0 ^{bcde}	1,200 ^a	0.17 ^c
	MS+2ip	2.3 ^c	5.9 ^a	13.9 ^a	800 ^b	0.18 ^c
	VS+2ip	2.4 ^c	5.1 ^b	10.7 ^{abc}	1100 ^a	0.27 ^a
	WPM+2iP	2.8 ^b	3.8 ^c	11.2 ^{ab}	800 ^{bc}	0.15 ^c

Different letters show significant difference in each column according to Duncan's Multiple Range Test ($P \leq 0.05$).

Note: MS – Murashige & Skoog 1962; VS – van der Salm *et al.* 1994; WPM – Woody Plant Medium; 2ip – N⁶-(2-Isopentenyl) adenine.

spectively and the highest amount of dry weight (0.27 mg) was observed in 'Golbahar' cultured in VS medium containing 2ip.

The recommended medium for each cultivar based on the data presented in Table 3 and general quality of the explants (without data) was as follow: 'Golbahar' (MS), 'Soltani Shabestari' (MS + 2ip), 'Sharbati' (MS), 'Golden Delicious' (WPM or MS + 2ip). Figure 2 shows the explants at the proliferation stage (different media) tested for the four cultivars. The roman numerals highlighted in red and underline, show the best culture medium for each cultivar. The influence of shoot culture media types on the quality and quantity of growth of *Ocimum basilicum*

has been reported by Monfort *et al.* (2018). Arab *et al.* (2014) investigated the interaction between media and growth regulators in G × N15 (a hybrid of almond × peach) and reported that the best medium was MS supplemented with 1 mg/L BAP. A strong influence of the culture media mineral composition on the micropropagation and the nutritional status of *Eucalyptus dunnii* cultures was also reported by Oberschelp and Gonçalves (2016). They stated that variability introduced by each genotype deterred the detection of additional effects. Hence they were able to develop a basal media based on the mineral composition of tissues of the species and optimized it for specific clones in order to adjust the nutritional

Figure 2. Effect of different media on explant proliferation of apple cultivars: I = MS, II = VS, III = WPM, IV = MS + 2IP, V = VS + 2IP, VI = WPM + 2IP. The roman numerals highlighted in red and underline show the best culture medium for each cultivar. Note: MS – Murashige & Skoog 1962; VS – van der Salm *et al.* 1994; WPM – Woody Plant Medium; 2ip – N6-(2-Isopentenyl) adenine.

requirements for each genetic material. Accordingly, an interaction effect of various culture media, in terms of their composition, and different apple cultivars was revealed at current work, though all cultivars reacted favorable to micropropagation in MS medium. However, the presence or absence of cytokinin 2ip in the culture media showed significant and incremental improvements in growth indices. Schaller *et al.* (2015) reviewed the role of cytokinin and auxin interaction in plant development and reported that in shoot apical meristem (SAM) a group of initial stem cells surrounds a small group of mitotically less active cells, known as the organizing center (OC). The cells of the OC are located at the base of a region with a low rate of cell division

called the central zone (CZ). Cells moving from the CZ into a region with a higher rate of cell division named the peripheral zone (PZ), give rise to the axillary shoots. The OC of the SAM is the site of maximal cytokinin activity, rather than of auxin activity. They concluded in the SAM cytokinins promote the proliferation of undifferentiated cells, while auxins act in the PZ to induce cellular differentiation and organ outgrowth. However, the type and concentration of the cytokinin needed for each genotype even within a species is variable, which is in agreement with our results.

Optimizing rooting stage using mycorrhiza *P. indica*

The results of the study indicated that rooting percent, the number of roots/explant, total root length/

Figure 3. Comparison of the interaction between cultivar and type of medium on A) rooting percent, B) average number of roots/explant, C) total length of roots /explant [cm], D) average root thickness [cm] of different apple cultivars. R1: MS, R2: LAM, R3: HAM, R4: MS + *P. indica*, R5: LAM + *P. indica*, R6: HAM + *P. indica*.

Note: MS – Murashigie & Skoog 1962; LAM – low auxin medium; HAM – high auxin medium.

explant, and thickness of the roots in the three cultivars ('Sharbati', 'Soltani Shabestari', and 'Golden Delicious') were significantly affected by rooting treatments. In all cultivars, MS medium containing either low or high auxin concentrations (LAM & HAM) for three weeks followed by a treatment of MS medium containing *P. indica* for another three weeks (treatments R5 & R6) increased the quality and quantity of root production. Figure 3A illustrates that maximum rooting percent in 'Sharbati' (40%) and 'Soltani Shabestari' (80%) were obtained in treatments R5 and R6 respectively. Whereas, the highest percent of rooting (80%) in 'Golden Delicious' was attained in R5. The maximum number of roots/explant in 'Sharbati' (3.5) and 'Golden Delicious' (8.87) were observed in treatment R5, but the maximum number of roots/explants in 'Soltani

Shabestari' (10.75) was attained in R6 (Figure 3B). The highest total length of roots/explant in 'Sharbati' (18.75 cm), 'Soltani Shabestari' (6.7 cm) and 'Golden Delicious' (11 cm) was observed in treatment R5 (Figure 3C). The greatest root thickness in 'Sharbati' (1.35 cm) and 'Soltani Shabestari' (1.17 cm) were detected in R5, but in 'Golden Delicious' maximum root thickness (1.3 cm) was observed in R2 (Figure 3D). Figure 4 and Figure 5 illustrate the prototype plantlets at rooting and acclimating stages of micropropagation respectively. The figures indicate that in 'Sharbati' and 'Golden Delicious' using fungus *P. indica* in combination with auxins resulted in strong plantlets with substantial amounts of roots. However, in 'Soltani Shabestari' although MS basal medium also resulted in the emergence of roots the plantlets from treatments

Figure 4. Effect of different treatments at rooting stage of three cultivars ('Sharbati', 'Soltani Shabestari' and 'Golden Delicious'). Note: MS – Murashigue & Skoog 1962; LAM – low auxin medium; HAM – high auxin medium.

containing *P. indica* grew stronger and healthier compared to the ones that excluded *P. indica*.

In this investigation, a combination of two types of auxins (IBA and NAA) rather than one type of auxin was used at the rooting stage. Yusnita *et al.* 2018 showed that in Malay apple cuttings, a combination of IBA and NAA was more effective on rooting than NAA or IBA alone. They reported that application of IBA+NAA, caused greater root length and the adventitious root morphologies were more branched and more fibrous, compared to the unbranched cuttings treated with NAA or IBA. Moreover, Yusnita *et al.* 2018 illustrated that combination of the two auxins (IBA and NAA) increased shoot sprouting percent, shoot length, and number of leaves per shoot.

The endophytic fungus *P. indica* is a symbiotic microorganism that has beneficial effects on the

host plants such as; growth promotion, development of the root systems, and increases tolerance to abiotic stresses (Lahrmann & Zuccaro 2012). Shah *et al.* (2019) investigated the growth pattern (number and length of roots and shoots) and various metabolites of *in vitro* propagated *Cymbidium aloifolium* plantlets that interacted with the *P. indica*. They identified growth regulators such as auxin, ascorbic acid, andrographolide, hexadecanoic acid, and DL-proline and reported *P. indica*-colonized plantlets showed improved growth rate compared to un-colonized control plantlets. In another study, *P. indica* plugs (P-1) and a *P. indica* chlamydospore suspension (P-2) were co-cultivated with banana seedlings at the rooting stage and the results indicated that P-2 treatment was more effective than the P-1 treatment on plant height and root length in the

Figure 5. Effect of different treatments at rooting stage on the acclimation stage of three cultivars ('Sharbati', 'Soltani Shabestari' and 'Golden Delicious'). Note: see Figure 4.

first 3 weeks (Li *et al.* 2019). The strong potential of *P. indica* as bio-fertilizer for ornamental plant cultivation has been demonstrated by Lin *et al.* (2019).

When biochemical analysis of the base biosynthesis pathways for the production of auxin was carried out the results indicated that *P. indica* can induce phytohormones via Indole-3-Pyruvic acid to produce indole 3-acetic acid and Indole-3-lactate (Hilbert *et al.* 2012). Sirrenberg *et al.* (2007) reported that co-cultivation of *P. indica* with *Arabidopsis* resulted in highly branched root systems and concluded that this effect was due to the diffusible ability of *P. indica* which could be mimicked by IAA. Moreover, they showed that *P. indica* produced IAA in liquid culture and suggested that auxin production affecting root growth is responsible for, or at least contributes to, the beneficial effect of *P. indica* on its host plants. In our study MS basal culture medium containing *P. indica* did not induce rooting, however, when MS media was enhanced by auxins (NAA and IBA) root system developed and acclimation of plantlets was successful.

CONCLUSIONS

The results of this study showed that both genotype and season affected the survival rate and contamination caused by bacteria and fungi at the establishment stage and maximum percent of survival was obtained in explants collected in spring. At the proliferation stage, all cultivars reacted favourable to micropropagation in MS medium, but the presence or absence of cytokinin 2ip in the culture media showed significant and incremental improvements in growth indices. Rooting and acclimation stages were significantly enhanced when plantlets were treated with media containing auxins followed by media that included the mycorrhiza fungus *P. indica*.

Acknowledgements. This work was funded by the Agricultural Biotechnology Research Institute of Iran (ABRII) and Temperate Fruits Research Center (Project no. 34-05-0533-960261). The authors report no potential conflict of interest and believe they have followed the ethical standards in conducting the research and preparing the manuscript.

REFERENCES

- ARAB, M.M. – YADOLLAHI, A. – SHOJAEIYAN, A. – SHOKRI, S. – GHODJAH, S.M. 2014. Effects of nutrient media, different cytokinin types and their concentrations on *in vitro* multiplication of G × N15 (hybrid of almond × peach) vegetative rootstock. In *Journal of Genetic Engineering and Biotechnology*, vol. 12, no. 2, pp. 81–87.
- BATELJA LODETA, K. – VUJEVIĆ, B. – ŽANETIĆ, M. – GUGIĆ, J. – OČIĆ, V. – ŠAKIĆ BOBIĆ, B. – BENČIĆ, Đ. – BOŠNJAK MIHOVILOVIĆ, A. – HABUŠ JERČIĆ, I. – ČMELIK, Z. – KEREŠA, S. 2019. Evaluation of morphological and chemical characteristics and micropropagation of traditionally grown domesticated apple varieties in Croatia. In *Journal of Central European Agriculture*, vol. 20, no. 1, pp. 274–291.
- BHATTACHARYA, A. – SINGH, R. 2018. Optimization of micropropagation from nodal segments of apple (*Malus × domestica* Borkh) cultivars golden delicious and red Fuji. In *Current Journal of Applied Science and Technology*, vol. 31, no. 3, pp. 1–9.
- DONG, S. – TIAN, Z. – CHEN, P.J. – SENTHIL KUMAR, R. – SHEN, CH. – CAI, D. – YEH, K.W. 2013. The maturation zone is an important target of *Piriformospora indica* in Chinese cabbage roots. In *Journal of Experimental Botany*, vol. 64, no. 14, pp. 4529–4540.
- DRUEGE, U. – BALTRUSCHAT, H. – FRANKEN, P. 2007. *Piriformospora indica* promotes adventitious root formation in cuttings. In *Scientia Horticulturae*, vol. 112, no. 4, pp. 422–426.
- FAO. 2016. Available on URL; <http://www.Fao.Org>.
- FRANKEN, P. 2012. The plant strengthening root endophyte *Piriformospora indica*: potential application and the biology behind. In *Applied Microbiology and Biotechnology*, vol. 96, no. 6, pp. 1455–1464.
- GENG, F. – MORAN, R. – DAY, M. – HALTEMAN, W. – ZHANG, D. 2016. Increasing *in vitro* shoot elongation and proliferation of 'G. 30' and 'G. 41' apple by chilling explants and plant growth regulators. In *HortScience*, vol. 51, no. 7, pp. 899–904.
- HILBERT, M. – VOLL, L.M. – DING, Y. – HOFMANN, J. – SHARMA, M. – ZUCCARO, A. 2012. Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots. In *New Phytologist*, vol. 196, no. 2, pp. 520–534.
- HOHTOLA, A. 1988. Seasonal changes in explant viability and contamination of tissue cultures from mature Scots pine. In *Plant Cell, Tissue and Organ Culture*, vol. 15, no. 3, pp. 211–222.
- HUA, M.D.S. – KUMAR, R.S. – SHYUR, L.F. – CHENG, Y.B. – TIAN, Z. – OELMÜLLER, R. – YEH, K.W. 2017. Metabolomic compounds identified in *Piriformospora indica*-colonized Chinese cabbage roots delineate symbiotic functions of the interaction. In *Scientific Reports*, vol. 7, no. 1, pp. 9291.
- JAFARKHANI KERMANI, M. – HOSSEINI, Z.S. – HABASHI, A.A. 2009. A refined tissue culture medium for *in vitro* proliferation of apple rootstocks. In *Acta Horticulturae*, no. 829, pp. 313–318. DOI: 10.17660/ActaHortic.2009.829.48.
- KAUR, A. – SINGH, D. – GUPTA, N. – KUMAR, A. 2018. *In vitro* propagation of important rootstocks of apple for rapid cloning and improvement. In *Biotechnologies of Crop Improvement*, vol. 1, pp. 215–241.
- LAHRMANN, U. – ZUCCARO, A. 2012. Opprimo ergo sumevasion and suppression in the root endophytic fungus *Pir-*

- iformospora indica*. In *Molecular Plant-microbe Interactions*, vol. 25, no. 6, pp. 727–737.
- LI, D. – MENSAH, R.A. – LIU, F. – TIAN, N. – QI, Q. – YEH, K. – LAI, Z. 2019. Effects of *Piriformospora indica* on rooting and growth of tissue-cultured banana (*Musa acuminata* cv. *Tianbaojiao*) seedlings. In *Scientia Horticulturae*, vol. 257, pp. 108649.
- LIN, H.F. – XIONG, J. – ZHOU, H.M. – CHEN, C.M. – LIN, F.Z. – XU, X.M. – YEH, K.W. 2019. Growth promotion and disease resistance induced in *Anthurium* colonized by the beneficial root endophyte *Piriformospora indica*. In *BMC Plant Biology*, vol. 19, no. 1, pp. 40.
- LIU, H. – SENTHILKUMAR, R. – MAM G. – ZOU, Q. – ZHU, K. – SHEN, X. – YEH, K.W. 2019. *Piriformospora indica*-induced phytohormone changes and root colonization strategies are highly host-specific. In *Plant Signaling and Behavior*, vol. 14, no. 9, pp. 1–13. DOI: 10.1080/15592324.2019.1632688.
- MONFORT, L.E.F. – BERTOLUCCI, S.K.V. – LIMA, A.F. – DE CARVALHO, A.A. – MOHAMMED, A. – BLANK, A.F. – PINTO, J.E.B.P. 2018. Effects of plant growth regulators, different culture media and strength MS on production of volatile fraction composition in shoot cultures of *Ocimum basilicum*. In *Industrial Crops and Products*, vol. 116, pp. 231–239.
- MOZAFFARIAN, V.A. 1996. *Dictionary of Iranian plant names* (Latin, English, Persian). Farhang Moaser Publishers. ISBN 964-5545-40-4.
- OBERSCHHELP, G.P.J. – GONÇALVES, A.N. 2016. Assessing the effects of basal media on the *in vitro* propagation and nutritional status of *Eucalyptus dunnii* Maiden. In *In vitro Cellular and Developmental Biology-Plant*, vol. 52, no. 1, pp. 28–37.
- SCHALLER, G.E. – BISHOPP, A. – KIEBER, J.J. 2015. The yin-yang of hormones: cytokinin and auxin interactions in plant development. In *The Plant Cell*, vol. 27, no. 1, pp. 44–63.
- SHAH, S. – THAPA, B.B. – CHAND, K. – PRADHAN, S. – SINGH, A. – VARMA, A. – PANT, B. 2019. *Piriformospora indica* promotes the growth of the *in-vitro*-raised *Cymbidium aloifolium* plantlet and their acclimatization. In *Plant Signaling and Behavior*, vol. 14, no. 6, pp. 1596716.
- SHAKYA, M. – GOTTEL, N. – CASTRO, H. – YANG, Z.K. – GUNTER, L. – LABBE, J. – PODAR, M. 2013. A multi-factor analysis of fungal and bacterial community structure in the root microbiome of mature *Populus deltoides* trees. In *PLoS One*, vol. 8, no. 10, pp. e76382.
- SIRRENBERG, A. – GOBEL, C. – GROND, S. – CZEMPINSKI, N. – RATZINGER, A. – KARLOVSKY, P. – SANTOS, P. – FEUSSNER, I. – PAWLOWSKI, K. 2007. *Piriformospora indica* affects plant growth by auxin production. In *Physiologia Plantarum*, vol. 131, no. 4, pp. 581–589.
- YUSNITA, Y. – JAMALUDIN, J. – AGUSTIANSYAH, A. – HAPSORO, D. 2017. A combination of IBA and NAA resulted in better rooting and shoot sprouting than single auxin on malay apple [*Syzygium malaccense* (L.) Merr. & Perry] stem cuttings. In *AGRIVITA, Journal of Agricultural Science*, vol. 40, no. 1, pp. 80–90.

Received: June 28, 2020
Accepted: December 14, 2020